LABORATORY MANUAL OF GENERAL PHYSIOLOGY

BARNES

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Laboratory Manual of General Physiology

BY THE SAME AUTHOR

Textbook of General Physiology

References to this book, in the "Laboratory Manual," are indicated by the word "Text"

Laboratory Manual of General Physiology

By **T. Cunliffe Barnes,** D. Sc.
Assistant Professor of Biology,
Yale University

Philadelphia
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(The number of experiments is indicated after each topic.)

LABORATORY MANUAL OF GENERAL PHYSIOLOGY

PREFACE

The following experiments represent a year's laboratory work in the elementary general physiology of animals. The subjects treated and the sequence are obviously matters for each instructor to decide. Elaborate quantitative experiments, so essential for a thorough understanding of general physiology, are not included as these depend largely on the equipment available in particular laboratories. Animal behavior is treated first, owing to the availability of living material in October; and the experiments serve to direct attention to the living organism. This is important inasmuch as the next five topics are largely "test tube" experiments. When students are thoroughly prepared in physical chemistry this preliminary work on physical and chemical principles can be considerably abbreviated. The next five topics deal largely with irritability or the response of the organism to the external environment. Topic XI, dealing with the permeability of living cells, might be studied earlier in the course. Experience suggests, however, that it is desirable to return to a consideration of first principles after an interval of gross descriptive physiology, and the permeability experiments afford a lucid interval which breaks the monotony of the kymographic routine in connection with the study of muscular contraction and cardiac rhythm. The last five topics (XII–XVI) deal with events occurring inside the organism, i.e., the "milieu intérieur" in the sense of Claude Bernard. Topic XII, Temperature Characteristics, affords an appropriate transition between the study of external and internal factors. Through the quantitative analysis of the effects of an environmental factor (temperature), some insight into the internal regulation of function is obtained. The experiments on respiration are reserved for the end of the course when the collecting season re-opens. It is not intended that all of the experiments in each topic be performed (i.e., in VI, IX, XI and XIII the essential principles involved can be illustrated by a judicious selection of experiments). Owing to the general interest in vertebrates the experiments involving invertebrate material are placed at the end of each section. Illustrations are reduced to a minimum since diagrams cannot replace actual demonstrations of apparatus. References to the literature are

intended for advanced students who may wish to make a special study of a particular experiment. The writer will be glad to receive criticisms and suggestions concerning any of the suggested procedures. In every case directions have been revised while working with beginning students.

No originality can be claimed for an elementary manual of this sort. Many experiments have been taken from laboratory outlines developed by Professor W. J. Crozier. For the muscle, heart and blood experiments it is impossible to improve on the excellent directions given in Professor W. B. Cannon's "Laboratory Course in Physiology" (Harvard University Press). Several experiments have been adapted from Professor E. Newton Harvey's "Laboratory Directions in General Physiology" (Princeton University Press, Third Revised Edition, co-author A. K. Parpart, Henry Holt and Co.) and Professor C. G. Rogers' "Laboratory Outlines in Comparative Physiology" (McGraw-Hill Book Co., Inc.). The writer wishes to acknowledge valuable suggestions received from Mr. E. J. Larson.

References to the author's "Textbook of General Physiology" are indicated by the word "Text" followed by the page number.

I. ANIMAL BEHAVIOR

1. Locomotion of the Earthworm.—Study the creeping movements on a moist glass plate. Observe the alternating waves of contraction of the circular and longitudinal muscles (Peristalsis). For general accounts of coordination in the earthworm, cf. Baglioni, in Winterstein's "Handbuch vergl. Physiol.," vol. 4, pp. 113–119, 123–126, 1913; Rogers, "Comp. Physiol.," pp. 491–515, 1927; von Buddenbrock, "Grundriss vergl. Physiol.," pp. 221–226, 1928.

2. Influence of the Brain on Locomotion.—Cut the ventral nerve cord of a worm near the middle of length, injuring the body wall as little as possible. Note carefully the effect, if any, on the coordination in locomotion. Cf. Rogers, "Comp. Physiol.," p. 512. Does the brain determine the coordination? In another worm, in the same spot, cut through everything, EXCEPT the nerve cord, and compare the coordination. For a description of nervous system see Prosser, Quart. Rev. Biol., 9: 181, 1934.

3. Mechanics of Coordination (Friedlander's Experiment) (Pflüger's Arch., 58, 1894).—Cut an earthworm into two parts, and with a needle sew them together again. How is coordination between the anterior and posterior pieces now effected? (cf. von Buddenbrock, Grundriss der vergl. Physiol., p. 222). Note that the contraction wave is not blocked. For a critical appraisal of Friedlander's results cf. van Essen, Zeit. vergl. Physiol., 15: 389, 1931; Maloeuf, Biol. Zentralblatt, 56: 379, 1936.

4. Phototropism.—Examine the phototropic orientation of the earthworm creeping on the surface of moist filter paper when exposed to one source of light. Cf. Parker, Am. J. Physiol., 5: 151, 1901. Repeat with

worms from which the first (about ten) segments of the body have been removed.

5. Stereotropism (Tactile response).—Examine the stereotropic response when the animal is creeping in lateral contact with a glass plate. Turn the earthworm on its dorsal surface. Describe result. How could you show that the righting response is due to stereotropism? Try it and describe the result. The inverted worm may be suspended by two threads. Focke (Zeit. wiss. Zool., 136: 376, 1930) claims the righting reaction is not a gravity effect.

6. Chemical Sensitivity.—Test response of the earthworm to local application of a drop of M/10 KCl. What region of the body is

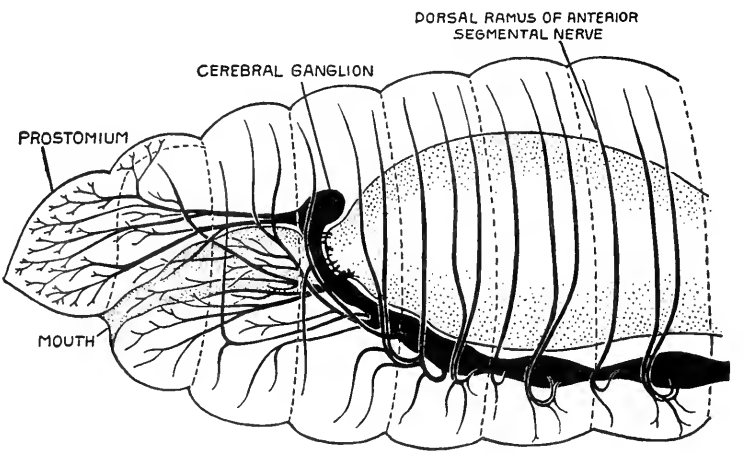

Fig. 1.—Relative positions of the brain and ventral nerve cord in the earthworm (after a drawing by Prof. W. N. Hess in Roger's Textbook of Comparative Physiology, McGraw-Hill). Focke (Zeit. wiss. Zool., 136: 385, 1930) describes how the brain may be removed by a hooked wire inserted by way of the mouth.

most sensitive? Arrange several regions of the body in order of their sensitivity.

7. Galvanotropism (Orientation by electric current).—Place a worm on a moist glass plate and pass current from one dry cell **transversely** through the middle segments by means of non-polarizable electrodes made of glass tubing containing Ringer's fluid and plugged with cotton. Do the ends turn toward the cathode? Carefully sever the nerve cord on each side of the stimulated region and repeat the experiment (cf. Moore, J. Gen. Physiol., 5: 453, 1923).

8. Influence of the Brain on Sensitivity.—Narcotize a worm slightly with chloretone. Carefully cut through the dorsal body wall of the third somite. (Fig. 1.) With a hot needle destroy the cerebral hemi-

spheres. Avoid injury to other parts. Place the worm for a few minutes in water to recover from the anesthetic.

Repeat Exps. 4, 5, and 6. Has sensitivity diminished? Will the worm spontaneously burrow in earth? Place the anterior end under earth.

9. Homostrophic Reflex (By which the anterior and posterior ends are kept parallel).—Displace to one side the tail end of an earthworm which is creeping in a straight line. Observe the orientation of the head end. Repeat with a worm lacking the first 20 segments.

10. Inhibition of Homostrophic Reflex by Stereotropism.—Allow a worm to crawl in contact with a glass plate. As it rounds the corner remove the plate and observe the homostrophic reflex released with contact removed. Meal worms (Tenebrio) may be used if available. Cf. Crozier, J. Gen. Physiol., 5: 597, 1923.

11. Evidence for Reciprocal Inhibition.—Inject a worm with strychnine sulphate 1/10,000 and compare its locomotory movements with those of a normal worm. Avoid injecting alimentary canal (cf. Knowlton and Moore, Am. J. Physiol., 44: 490, 1917).

12. Nerve Plexus.—Cut out a section, about 20 segments, from the middle of a worm. Carefully remove the alimentary tract and ventral nerve cord. Any spontaneous movement? Stimulate with a needle and with a weak electrical shock. Does conduction travel in all directions?

13. Muscle Tension.—Anesthetize cutaneous receptors of a worm by immersion in M/8 $MgCl_2$ until gentle stroking with a brush elicits no response. Make a muscle preparation from the mid-body region and connect to a light lever writing on a kymograph as shown in demonstration. Does the muscle make spontaneous contractions? Now weight the muscle with 1 or 2 grams and record contractions (cf. Moore, J. Gen. Physiol., 5: 327, 1922). If this experiment does not work use the preparation for a smooth muscle record on the kymograph. Stimulate electrically with fine wires.

14. Stereotropism in Isopods.—Allow a sow bug to crawl with one surface in contact with a glass plate and observe the direction of turning when the plate is passed. Place the isopod so that it crawls between the edges of two plates and observe the subsequent direction. Repeat but remove one plate just before the animal reaches the end of the passage (cf. Crozier, J. Gen. Physiol., 6: 531, 1924).

15. Chemical Trap Action of Jennings.—Place a drop of M/80 HCl on a glass plate containing a thick suspension of Paramecium. Observe the edges of the drop. Place a drop of 0.02% acetic acid containing Paramecium on a slide of culture medium. Do the animals stay in the acid? **To prove that these reactions are not chemotropic,** proceed as follows. Fill four capillary tubes with (1) culture medium, (2) distilled water, (3) M/180 HCl, (4) 0.02% acetic acid. Introduce the ends into a large

drop of culture medium containing Paramecium and later examine the tubes under the microscope or with a large reading glass for Paramecia. Make sure that there are no air bubbles at the tips of capillary tubes. The tubes may be held with plasticine at the edge of the culture fluid. Cover to prevent evaporation (cf. Loeb, "Forced Movements," pp. 144–146, 1918).

16. Geotropism (Orientation by Gravity) in Ants.—With a soft pencil carefully trace the path followed by an ant crawling over a sheet of paper on a glass plate tilted at 5°, 25°, 45°, 80°, etc. from the horizontal; 5–10 trails at each inclination should be recorded. The angle of orientation (θ) is that between the path and the transverse axis of the plane. Plot mean angles of orientation against sine α(α = angle of tilt of the plane).

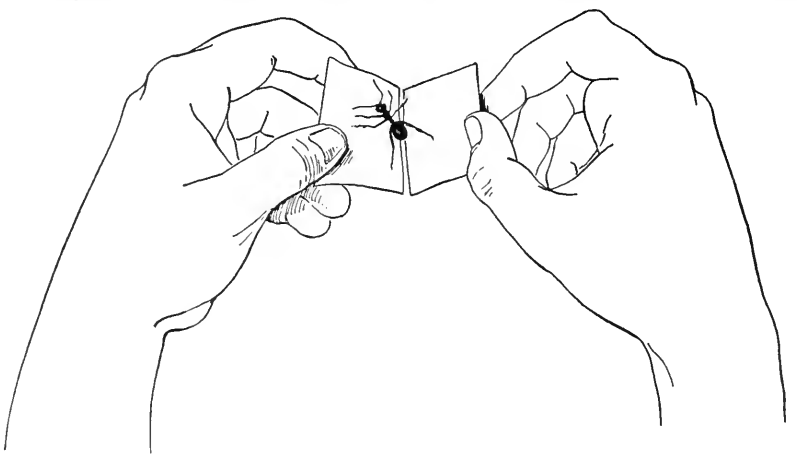

Fig. 2.—Method of handling ants and other small animals.

Ants must be handled with great care by means of two small paper squares on which the ant crawls and is placed on the plane (see Fig. 2). At low α angles random movements occur in all directions but statistically the θ angles increase with the steepness of the plane. Cf. Barnes, J. Gen. Psychol., 2: 517, 1929.

17. Phototropism in Limax.—Place a slug with one eye removed (on the previous day) on a moist plate under bright overhead illumination. Observe the circus movement. To which side does the animal turn? Explain. Animals must be kept in the dark immediately prior to experiment (cf. Crozier in Murchison's "Handbook of General Experimental Psychology," p. 12, 1934).

18. Orientation between Opposing Beams of Light.—Allow a meal worm or pill bug to crawl between two lights set up in a special dark room. Record the paths of orientation at various distances between the lights. With an illuminometer calibrate the distance in centimeters

between the lights with the intensity in foot candles. Record the paths at several distances between the lights. Cf. Crozier, J. Gen. Physiol., 8: 671, 1927.

19. Tonic Immobility. (Animal hypnosis).—Place a newt dorsum down on a flat surface and observe the induced state of immobility. Will limbs remain in any position if moved? Observe breathing. Determine the average duration of immobility. Record the temperature (cf. Hoagland, J. Gen. Physiol., 11: 715, 1928; Steiniger, Ergebnisse Biol., 13: 348, 1936).

II. SURFACE TENSION

For quantitative methods, see Dorsey, Bureau of Standards Scientific Papers, No. 540, vol. 2, p. 563, 1926; Duclaux, Capillarité, 1934. Text p. 50.

1. Floating Needle.—Draw a needle between thumb and finger to cover it with oil. Carefully drop needle on surface of water. Note depression of water film and explain why needle floats. Now clean the needle with Na_2CO_3 solution and repeat. Explain result (cf. Descartes, 1638, Les Météores, Oeuvres vol. v, p. 187; Adam, "Physics and Chemistry of surfaces," 1930, p. 191). Text p. 49.

2. Submerged Ring.—A weighted cork supports a large wire ring in the air above water in a deep jar. Submerge the figure and note that its rise is arrested when the ring reaches the surface. (Surface tension figures can be obtained from the Central Scientific Co.)

3. Van der Mensbrugghe's Experiment.—Dip a wire ring with an attached small loop of thread into soap solution to form a film that holds the loop of thread. Puncture the film inside the thread loop with a hot wire. Explain result (cf. Bayliss, p. 48).

4. Maxwell's Example of Surface Tension.—Secure a rectangular wire frame of which one side can slide along the two adjacent sides Dip in soap solution to secure film. Hold frame horizontally and slightly approximate adjacent sides to allow slide wire to move freely. Explain result (Scarth and Lloyd, "Gen. Physiol," p. 21).

5. Minimal Area Configurations.—Dip a cubical wire frame into soap solution. Try dipping several times in succession. Sketch geometrical patterns formed. Explain. Best results are obtained with Plateau's solution (cf. C. V. Boys, "Soap Bubbles," p. 170, 1931).

6. Bubbles.—Compare the shape of (a) a soap bubble freely floating in air; (b) the same compressed or pulled out (hanging from pipe); (c) two equal sized bubbles resting on soap solution in contact with one another; (d) unequal bubbles in contact. Place a small bubble in contact with a large one in a Petri dish of soap solution. How does the internal pressure vary with the size of the bubble? To show the law of constant mean curvature draw out a bubble between two clay pipes (cf. Scarth and

Lloyd, p. 20;. Lawrence, "Soap Films," 1929). Sketch shapes of bubbles in each case. Text p. 50.

7. Retreating Film.—Make a film of soap solution over the wide end of a funnel; close the end of the stem with finger; remove finger. Result? Explain. Why does the film move downward?

8. Limnological Example of Surface Tension.—Place a living water-strider (Gerris) on a water surface. Add a drop of oil and note change of posture (side view). This experiment was suggested by Prof. G. Evelyn Hutchinson. For the ecology of the surface film cf. Needham and Lloyd, "Life of Inland Waters," p. 327, 1916.

9. Rapid Changes in Surface Tension.—Drop a few grains of camphor on water. Explain movement (cf. Scarth and Lloyd, p. 201).

10. Internal Pressure in Liquid Drops.—Water is drawn up into a vertical capillary tube, then allowed to drop until equilibrium. Raise a dish of water until it touches the pendant drop. The distance through which the meniscus falls measures the internal pressure due to surface tension of suspended drop (cf. Sentis, Journ, de Phys., 3e. Ser. 6: 183, 1897).

11. Crossing the Red Sea.—Draw a brush dipped in alcohol across a layer of aqueous eosin on a glass plate. Explain the tangential movement of film (cf. Exodus, 14: 21).

12. Adhesion and Cohesion.—The molecular forces causing surface tension are concerned with cohesion, adhesion and solution. Dip glass into water, and into Hg. Is the glass wetted? Is the fluid in each case lifted against gravity? Test with a capillary tube. Place two glass slides together with a film of water between and try to separate. Explain. Experiment with the adhesion disc provided.

13. "Negative" Surface Tension.—Place a speck of lecithin on a microscope slide. Cover with a drop of N/100 HCl and a cover glass supported on glass splinters. Under microscope observe films of **maximum** area (at edge of lecithin). If possible observe in polarized light. In rare cases packing in film may give special molecules their position of greatest equilibrium (cf. Virchow's Archiv., 6: 571, 1854; Scarth and Lloyd, p. 26; Burns, "Biophysics," p. 109; Leathers, Lancet, 1925, 803; Needham, "Order and Life," p. 162, 1936). Text p. 167.

14. Surface Tension and Electrical Charge.—Dip wires from two dry cells (with rocking key in circuit) into opposite sides of a Petri dish containing a Hg drop between the electrodes. Close and open the key. Try reversing the current. Note movement of Hg relative to the poles. Explain (Christiansen, Ann. d. Physik., 4th ser., 12: 1072, 1903). Text p. 65.

15. Dead Space.—Mix approximately equal parts of molar sodium carbonate solution and half molar choral hydrate (about 3 cc. of each) in a test tube. Let the tube stand undisturbed. Within ten minutes

turbidity appears through the solution **except near the surface film.** Examine with eye level with meniscus and a light behind the tube. Do not disturb the tube. The reaction results in an increase of surface energy (opposed at the surface) (cf. Bayliss, p. 54). The water polymers in the film may also affect the reaction (cf. Barnes and Jahn, Quart. Rev. Biol., 9: 300, 1934).

16. Spreading of Film.—Sprinkle a little talcum powder on the surface of distilled water in a wide dish. Add a drop of oil. Explain. Obtain a tray of clean water. Make the surface visible with a little powdered sulphur. Add a strip of paper (a little shorter than the width of the tray). Add oelic acid to the water surface on one side of the strip. Result? (Cf. Langmuir, Jl. Chem. Education, 8:850, 1931.) Text p. 61.

17. Drop Method.—Fill a pipette to a given point with water. Slowly expel and count drops. Repeat with alcohol. Explain the difference.

18. Surface Tension Parabola.—With a stalagmometer determine the surface tension of solutions of alcohol of the following per cent by volume.

Per cent	20	40	50	60	70	80	90
Density	.976	.951	.93	.91	.89	.863	.833

Proceed with great care, making five determinations for each solution. Plot results with concentration as ordinates and surface tension (Dynes per cm.) as abscissae. Text p. 53.

$$\text{Surface tension} \frac{\text{No. drops } H_2O \times \text{Density of solution} \times 73}{\text{No. drops of solution}}$$

19. Surface Tension and Temperature.—With stalagmometer compare the surface tension of water at about 5° and 60° (cf. Kopaczewski, Protoplasma, 19: 262, 1933). Text p. 64.

III. IONS

1. Ionic Antagonism.—(These experiments are introduced here so that the results may be compared with later ionic effects.) Grow 4 wheat seedlings in each of the following mixtures of two salts in proportion by volume (0.1 M concentration):

(1) NaCl	100	95	90	50	25	0
$CaCl_2$	0	5	10	50	75	100

(2) A similar series of $MgCl_2$ vs. $CaCl_2$

(3) NaCl vs. KCl

(4) Controls in distilled water and diluted sea water (sea water is osmotically equivalent to 0.52 M NaCl)

Place 150 cc. of solution in a tumbler. Pour on melted paraffin. Puncture 4 holes with heated glass rod. Place roots in punctures. Wash seedlings carefully in distilled water and keep in distilled water

until used. **Avoid drying.** Measure in millimeters the three longest roots on each seed and record the average length per solution on label of culture with your name.

Measure again after four weeks. Plot the data as curves with average length as ordinates and composition of mixtures as abscissae (cf. Osterhout, "Injury, Recovery, and Death in Relation to Permeability," 1922; also Rubenstein, Protoplasma, 4: 259, 1928). Text p. 26.

HYDROGEN IONS

2. Acidity Determined by Means of Taste.—Place the following N/10 acids in their order of sourness: hydrochloric, acetic, and boric. Dry the side of the tongue and apply a small quantity of acid with a camel's hair brush. Rinse the mouth after each application. Dilute a sample of each acid ten times and repeat the experiment.

3. Acidity Determined by Hydrolysing Power.—Make an iodine spot plate determination of the prepared starch solution before beginning experiment. Place 3 cc. of the starch solution in each of three test tubes. Add 5 cc. of the N/10 acids to the test tubes. Raise all three to boiling and boil for one minute in a water bath. Remove simultaneously and cool quickly in tap water. Make the spot plate test. What is the order of hydrolysis? Differentiate between "total acidity" and "hydrogen ion concentration."

4. Action of Buffers.—Titrate 20 cc. of the peptone solution with N/10 NaOH in steps of 0.5 cc. of the NaOH. As each 0.5 cc. of the base is run in (stirring well) remove 5 drops of the solution to a spot plate and add indicator. Begin with Brom thymol blue using the Clark's color chart. As the pH increases use Phenol red and Cresol red in turn. Determine pH for each step. Plot results, pH on ordinate; cc. of base on abscissa.

5. Repeat the procedure in 4 using 20 cc. of distilled water in place of peptone solution and running the NaOH in steps of 0.2 cc. in place of 0.5 cc. Plot results upon the same axes as in 1. Explain difference between curves 1 and 2. Also criticize the experiment. Text p. 20.

6. Reaction between CO_2 and Buffers.—Prepare an alkaline buffer solution and introduce CO_2 from tank. Determine the pH. Repeat using distilled water. What is the pH of pure water?

Determination of pH.—Six unknowns (designated A-F) will be determined by indicators, Ex. 7, and if possible by potentiometric measurements, Ex. 8 (hydroquinone electrode).

7. The Indicator Method for Determining pH.—There are many variations of this. For rough determinations the spot plate and indicator chart are used alone (as in the experiment above). In more careful determinations the spot plate is used to ascertain the approximate pH range of the unknown. Then definite amounts of acid and basic buffer salts are com-

bined. The pH of these combined solutions has been carefully determined electrometrically. Combinations are made having a pH in the region of the unknown. Like amounts of indicator (10 drops) are then added to 10 cc. of the unknown and each one of the standards. The colors are matched in a comparator block. Sometimes the color of the unknown solution falls between that of two standards. The pH value is then interpolated.

When an unknown solution possesses a turbidity, which will prevent direct matching, the so-called "principle of Walpole" is used. This will be demonstrated. Text p. 20.

8. Potentiometric Method.—With the quinhydrone electrode determine the pH of the unknowns. Compare with results from indicator method. Text p. 21.

The theory of indicators is discussed by Clark, "The Determination of Hydrogen Ions." For mixtures of primary phosphate KH_2PO_4 and secondary phosphate Na_2HPO_4 and for McIlvaine's standards (Na_2HPO_4 and citric acid) see Table 1 on p. 110. The quinhydrone electrode is described by Clark, 2nd Ed., p. 289 and Michaelis, "Hydrogen Ion Concentration," 1926, p. 171. For the nature of the oxonium ion OH_3 cf. Barnes and Jahn, Quart. Rev. Biol., 9: 306, 1934.

IV. COLLOIDS

1. Adsorption.—(a) Add powdered charcoal to a solution of crystal violet in a test tube until color disappears. Filter off charcoal and wash it with acetone acidulated with a few drops of dilute HCl. Explain return of color.

(b) Dip filter paper in methylene blue solution (positive) and set aside. Dip another paper in eosin (negative). Wash both in distilled water. Compare permanence of stains. What charge does the paper bear?

2. Imbibition.—(a) Place 2 cc. granulated gelatin in each of six similar test tubes.

In No. 1 place 15 cc. distilled water
No. 2 place 15 cc. N/10 HCl
No. 3 place 15 cc. N/10 NaOH
No. 4 place 15 cc. N/5 NaCl
No. 5 place 15 cc. of equal parts N/20 HCl and N/10 NaCl
No. 6 place 15 cc. of equal parts N/20 NaOH and N/10 NaCl

Determine effect on imbibition by measuring rate of swelling. Shake after adding liquids. Observe effect of acid and alkali, and of salts alone and in presence of acid. Cf. Bayliss, p. 100. According to Pauli, dissociated salts of protein are formed by acid or alkali and the swelling is due to the affinity for water of the protein ion.

(b) Set up another series of six tubes with 2 cc. of granulated gelatin. Into the first place 15 cc. N/10 HCl; into next 15 cc. N/10 HCl diluted one half; next 15 cc. N/10 HCl diluted one fourth; next etc. Observe effect on imbibition.

3. Reversal of Emulsion.—Place 3 cc. of olive oil (colored with Sudan III) and 3 cc. of water into two test tubes. Place a crystal of $CaCl_2$ into one and a few crystals of NaCl into the other. Shake violently. Examine microscopically. Note dispersion of substances. Make several trials varying proportions of oil, water, and salt until good results are achieved. Consult Clowes' paper, Protoplasmic Equilibrium, J. Phys. Chem., 20: 407, 1916; Scarth and Lloyd, p. 156; Clayton, "The Theory of Emulsions," 1935. Text p. 187.

If time permits, determine the effect of electrical stimulation on the emulsions (cf. Dixon and Bennet-Clark, Proc. Roy. Dublin Soc., 20: 211, 1932).

4. The Tyndall Effect.—Project a powerful beam of light from some source into a small flask containing As_2S_3 sol and note whether the path of the beam becomes strongly luminous. Luminosity of the path of the beam is known as the Tyndall effect and indicates the presence of suspended particles (if no fluorescent material is present). Text p. 81.

Insert a Nicol prism between the light source and the colloidal suspension and rotate the prism. Note whether there is any change of luminosity as the Nicol is rotated. Cf. Robertson, "Optics," p. 273, 1929.

5. Solubility and the Colloidal State.—Pour 2 cc. of an acetone solution of a liquid fat into 100 cc. of water and get a suspension of minute particles of fat in water. It is a general principle that if A is soluble in B but insoluble in C, A will be thrown into suspension (often colloidal) when a solution of A in B is added to an excess of C (C and B being miscible).

6. Permeability Test for Colloids.—Make colloidal $Fe(OH)_3$ by pouring a concentrated solution of $FeCl_3$ into a beaker containing 100 cc. of boiling water. A rich red solution, which is very stable, is formed instantly. Place the colloidal suspension in a dialyzer. Test the dialysate for chloride ($AgNO_3$ test). Does the $Fe(OH)_3$ pass through? Keep the dialyzer set up for a week. Concerning dialysis (cf. Bayliss, p. 83).

7. Diffusion Test for Colloids.—Let solutions of Congo red, colloidal $Fe(OH)_3$, safranin, and fluorescein as well as $CuSO_4$ (10%) diffuse into jelly of 3% gelatin (use test tubes $\frac{2}{3}$ full of gelatin). Which are colloids as shown by speed of diffusion? For dimensions of particles in the colloidal state cf. Scarth and Lloyd, p. 156.

8. Hardy's Rule.—To 20 cc. portions of As_2S_3 add drop by drop solutions of NaCl, $BaCl_2$ and $AlCl_3$ provided. How many drops are necessary to produce a cloudy precipitate in each case? What is the relation between the valency of ions and coagulation? Consider the adsorption

isotherm (cf. Scarth and Lloyd, p. 160). For a general discussion of colloids, cf. Thomas "Colloid Chemistry," 1934. Text p. 84.

V. ENZYME ACTION

Peroxidase and "Oxidase."—Prepare potato juice by scraping the surface of potato with glass (not metal). Mix with an equal volume of water, stir, strain through cheese cloth. Divide into equal portions. Boil one for 5 minutes. In the tests use boiled solution as control in each case. To separate portions:

1. Add H_2O_2 and a few drops of gum guaiac solution (freshly prepared alcoholic solution) on a slide. Text p. 400.

2. Add guaiac solution alone. Compare with pulp from heart of potato. Cf. Bayliss, p. 584.

Catalase.—(Catalase is discussed by Meldrum, "Cellular Respiration," Chapt. IV, 1934. Stern has shown that catalase can attack organic peroxides (monoethyl hydrogen peroxide to acetaldehyde). Text p. 400.

3. To 5 cc. neutralized H_2O_2 in a test tube add 3 cc. crushed liver suspension. Result?

4. Repeat using liver previously boiled.

5. Repeat with

(a) 5 cc. fresh liver suspension plus 5 drops 10% HCl

(b) with 5 cc. liver suspension plus 1 cc. 1% $NaCO_3$ solution

(c) with liver suspension plus equal volume N/10 KCN

(d) with liver suspension plus equal volume saturated $HgCl_2$ solution

6. Dehydrogenase.—Slightly color with methylene blue two tubes of fresh unpasturized milk. Add a few drops of formaldehyde to one tube and observe any decolorization of the dye which occurs (cf. Wieland's book on "Oxidation," p. 57).

Try the same test with yeast suspension. Allow to stand. The yeast has H donors so addition of formaldehyde is unnecessary.

7. The Kinetics of the Decomposition of H_2O_2 by Catalase.—(The catalase extract can be prepared from potato or from liver. In the latter case the assistant should determine a dilution which will evolve oxygen at a rate suitable for measurement.)

Place a constant volume of catalase extract (i.e., 10 cc.) in an Erlenmeyer flask. By means of thread lower a small phial of H_2O_2 (upright) into the flask. Connect the flask with a burette arranged to collect the gas given off (the burette is filled with water and inverted over a finger bowl of water). Start by tipping over the phial. With constant shaking, record as frequently as possible the amount of gas given off. Determine if the speed of the catalase reaction increases in direct proportion to the amount of enzyme. Use several measured dilutions of catalase extract FROM THE SAME ORIGINAL SAMPLE. Vary also the concentration

of H_2O_2. In each case keep the volume of the mixture constant (with distilled water). Determine if the speed of the catalase reaction increases in direct proportion to the amount of enzyme; use several measured dilutions of potato juice from the same sample. Vary also the concentration of H_2O_2.

Compare times required to produce the same amount of change.

Repeat experiments immersing reaction-chamber (1) in ice water, (2) in water at 30°, (3) at room temperature. The flask should be immersed as far as possible in the water.

If possible, analyse the thermal effect. The kinetics of these catalase reactions are discussed by Northrop (J. Gen. Physiol., 7: 373, 1925; cf. also Williams, J. Gen. Physiol., 11: 309, 1928).

DIGESTION OF STARCH BY PANCREATIC AMYLASE

8. Effect of Concentration of the Substrate.—Measure into six clean test tubes the following proportions of soluble starch solution and distilled water; mix thoroughly.

Tube No.	1	2	3	4	5	6
H_2O, cc.	5	4	3	2	1	0
Starch solution, cc.	1	2	3	4	5	6

Now add 0.5 cc. of the pancreatic amylase solution to each test tube, mixing thoroughly and noting the time when the addition is made. Test for the rate of digestion by the iodine method. This consists in removing 5 drops from each mixture and adding a drop of the dilute iodine solution. During the course of digestion the color changes from blue through purple to red. This red stage is known as the erythrodextrin point. A stable solution of erythrodextrin is provided as a standard for colorimetric matching, thus sharpening the end point. Note the time interval from the beginning of the reaction to the erythrodextrin stage for each tube.

Plot the results (cf. Haldane, "Enzymes," p. 31, 1930). (Preparation of solutions. Add ¼ to ½ gram of pancreatin to 40 cc. H_2O. Shake thoroughly for at least five minutes. Filter through best quantitative filter paper. This may take 3 hours. Obtain a clear filtrate. Add filtrate to 500 cc. H_2O. Test sample with starch solution to obtain a reasonable digestion time. To prepare starch solution add 2 grams of soluble starch to 500 cc. H_2O. Boil 1 liter H_2O and add solution little by little. The solution must be clear and free from opalescence. Adjust solution so that equal parts of enzyme solution and starch solution digest in about 10 mins. at room temperature. Prepare standard erythrodextrin solution and fix by heating at right stage. Do not add iodine until time for comparison.)

9. Effect of Temperature upon Rate of Digestion.—Make up four tubes of soluble starch solution (3 cc. stock starch solution + 3 cc. H_2O in each). Place tube 1 in cracked ice; 2, in water bath at 15°C.; 3, in water bath at 30°C.; keep tube 4 at room temperature. Add 0.5 cc. of enzyme preparation to each. Observe time intervals for digestion by the same method as in the first exercise (cf. Gortner, "Outlines of Biochemistry," p. 727, 1929).

10. Effect of pH upon Rate of Digestion.—Place 3 cc. of stock starch solution in each of 4 test tubes. Add 3 cc. of Sørensen's phosphate mixture (pH = 5.288) to 1; 3 cc. mixture (pH = 6.239) to 2; 3 cc. mixture (pH = 7.168) to 3; 3 cc. mixture (pH = 8.043) to 4; add 0.5 cc. of enzyme solution to each; note digestion times. Then test the pH of each tube by the spot plate method to get the pH of each total mixture (cf. Gortner, p. 723, 1929).

Demonstration: Specificity of Enzyme Action.—Fermentation tubes, containing the same amount of yeast suspension but a different sugar in each, show the remarkable specificity of enzymes. The sugars used are: (1) glucose; (2) lactose; (3) galactose; (4) mannose; (5) xylose. Note how these sugars vary structurally. For specificity of enzymes, cf. Gortner, p. 528; Bayliss, p. 328; Mitchell, 2nd Ed., p. 492.

VI. PLASMOGENY

(PHYSICAL AND CHEMICAL MODELS OF LIVING SYSTEMS)

1. Mercury Amoeba.—Place a large drop of Hg in a watch glass of M/3 H_2SO_4. Place a crystal of K_2CrO_4 near the Hg and note amoeboid movements and "feeding" (cf. Bernstein, Pflüger's Arch., 80: 628, 1900). The interfacial tension of Hg is 375 dynes/cm. while that of a living amoeba is only 1–3 dynes/cm. (cf. Harvey and Marsland, Jl. Cell. Comp. Physiol., 2: 75, 1932). Text p. 171.

2. Ostwald's Physical Heart.—Place a large drop of Hg in a large watch glass (10 cm. diameter) of 2% HNO_3 (or 10% H_2SO_4). Clamp a sewing needle (with femur clamp on stand) so that the point just touches the Hg. Explain the rhythmic action due to the negatively charged carbon in the needle. Note odor of C_2H_2. Cf. Burns, "Biophysics," p. 49. To prevent cessation of pulsation by polarisation, a trace of K_2CrO_4 solution may be added (cf. Pohl, "Physical Principles of Electricity," p. 278, 1930). Text p. 190.

3. Mercury Octopus.—Place a large flat drop of Hg in a Petri dish of 40% H_2SO_4. Connect wires to a dry cell and draw out tentacles from the Hg with the positive pole. Now touch the octopus with the negative electrode. Explain movement (cf. Burns, Fig. 10). Text p. 190.

4. Formation of Foraminifera Shells.—Introduce a drop of a mixture of powdered glass and chloroform under water in a watch glass, or intro-

duce both separately. Observe that fine glass particles gather at the surface of the drop (cf. Rhumbler, Arch. Entwicklungsmech., 7: 283, 1898).

5. Cell Division (Animal).—Mix olive oil with chloroform until the mixture is heavier than water. Place a drop in distilled water. Allow N/3 NaOH to flow from two glass tubes one at either side of the drop or **apply a small crystal of NaOH at each end.** Explain the cleavage (cf. Gellhorn, "Lehrbuch allgemeine Physiol.," pp. 495–6, 1931; Gray, Quart. Jl. Micr. Sc., 66: 235, 1922). Text p. 171.

6. Cell Division (Plant).—Wet a fine thread with N/5 NaOH. Lay thread over middle of drop of oil-$CHCl_3$ mixture in distilled water. Try a coarse thread. Explain result. The thread represents the cellulose wall. A partition of minimal area, when two dimensions of a cell are small compared with a third as in a filament like Spirogyra must be transversely to the long axis (cf. D'Arcy Thompson, "Growth and Form," p. 358, 1917).

7. Biological Diffusion Patterns.—Pour a film of half saturated KNO_3 into a Petri dish resting on a white background. Allow a few drops of ¾ saturated KNO_3 containing eosin and India Ink (recently added) to fall on the film from heights of 0 to 4 ins. Observe the patterns which slowly unfold—ferns, neural folds, finger prints, etc. Leave undisturbed and proceed with the next experiments.

8. Mitosis.—Place a drop of half saturated KNO_3 on a glass plate resting on a white background. This represents the cytoplasm of the cell. In this carefully place a drop of the same solution deeply colored with India Ink (nucleus). On opposite sides of the drop place two drops of ¾ saturated KNO_3 stained with eosin. With a needle bring the drops in contact. Chromosome movements, asters, mitotic spindle, and alignment of chromatin on an equitorial plate may appear. Repeat 3 or 4 times (cf. Le Duc, "Mechanism of Life," Fig. 32).

9. Biological Theory of Brownian Movement.—Into a watch glass containing a mixture of equal parts of gasoline and olive oil drop 14% NaOH from a height of 60 cm. **Quickly** observe under microscope. Focus on small drops near surface. Explain movements (cf. Herrera, in Alexander's "Colloid Chemistry," vol. 2, p. 91, 1928). Note semblance to movements of bacteria.

10. Synthetic Colpoda.—Place a few drops of Herrera's solution B (water 100 parts, soda 14, rodamine 1) into a watch glass containing Herrera's Solution A (gasoline 100 parts, olive oil 50) and **quickly** observe under microscope stained forms. Observe cyclosis (cf. Beltram, Trans. Amer. Micros. Soc., 46: 69, 1927). Text p. 173.

11. Resin Model of Protoplasmic Streaming.—Add a drop of 14% NaOH to a mixture of gasoline 100 parts, olive oil 50 and resin 25 parts. **Quickly** observe under microscope. Focus on large yellow masses.

Observe streaming of granules. The movements soon subside. Repeat several times (cf. Herrera, in Alexander's "Colloid Chemistry," vol. 2, p. 85, 1928).

12. Cyclosis and Battle of the Protozoa.—(a) Place a drop of 14% NaOH containing India Ink into a watch glass containing equal parts gasoline and olive oil. Observe the protoplasmic streaming under the microscope. (b) With a rotary motion stir the NaOH rapidly with a needle, keeping the point touching the glass. Quickly observe under the microscope. Focus on the path made by the needle. Observe the amoebae fiercely gnawing each other in the line of battle.

13. Robertson's Camphor Amoeba.—Drop a mixture of 10% camphor in benzol on water on white background. Note movements. Explain (cf. Robertson, Science, vol. 36, p. 446, 1912). The fluttering of the margin resembles the macrophages in Lewis' films of tissue cultures (but with a different time scale). Text p. 173.

14. Synthetic Fungus.—Allow a drop of sodium silicate solution to fall from a height of 2 cm. into a film of concentrated HCl in a watch glass. Add a few cc. of distilled water. Note the form resembling a culture of bacteria or mold. Carefully observe under microscope. In a ring near the center will be found formations of **spores.** Formation of crystals may partially obscure result. The preparation is permanent and can be mounted on a slide in balsam. The HCl infiltrates through pores in the silica membrane (cf. Alexander's "Colloid Chemistry," vol. 2, p. 87; also Herrera, Memoria de la Sociedad Cientifica "Antonio Alzate," Mexico, 26: 44, 1907). Text pp. 164, 166.

15. Osmotic Growth.—Suspend by a thread a crystal of ferrocyanide in a test tube containing 3% $CuSO_4$ solution. Observe growth.

16. Galvanotropism of Artificial Plants.—Fill an upright specimen jar with 3% $CuSO_4$. At each end place electrodes of sheet copper connected to four dry cells in series (with key in circuit). Drop a few crystals of ferrocyanide into the jar. Place a light behind the jar. When upward growth begins turn on current. Toward which pole do the plants grow? (cf. Beutner, "Physical Chemistry of Living Tissues," Fig. 8, 1933).

17. Colony of Calcareous Sponges.—Place several small pieces of anhydrous $CaCl_2$ close together in a Petri dish containing Le Duc's solution (potassium carbonate 76 parts, sodium sulphate 20, tribasic potassium phosphate 4, all saturated). **Quickly** observe under microscope. Focus on outer edges of the clump of $CaCl_2$. Observe slow **streaming** into osmotic growths which becomes almost imperceptible after three minutes. After 10 mins. radial spicules appear. For typical sponges see Cambridge Natural History, vol. 1, Figs. 79 and 88. According to Gerarde's "Herbal" 1636, sponges are formed "of the foame or froth of the sea."

18. Liesegang Rings.—Pour a little 8.5% silver nitrate on gelatin gel in a test tube containing 0.1% potassium dichromate. Place in the ice-box for a day or two. Observe the rhythmic banding. Explain (cf. Scarth, and Lloyd, p. 161; Hedges and Myers, "The Problem of Physico-chemical Periodicity," p. 21, 1926). Text p. 161.

19. Epithelial Tissue.—Make a 10% solution of gelatin and pour in a Petri dish. When it has set place uniform drops of 5 or 10% ferrocyanide solution equally spaced at intervals of 5 mm. over the plate. Cover and set aside until next day. Observe the tissue formation (cf. Ponder, "Essentials of General Physiology," p. 108). Text p. 163.

CONTRACTILE SYSTEMS

VII. AMOEBOID MOVEMENT

1. Normal Movements.—Observe an amoeba in motion. Note direction of protoplasmic streaming. Compare with physical models of amoeboid motion previously studied. Could amoeboid movement cause cytoplasmic fission? (cf. Schaeffer, "Amoeboid Movement" 1920; Chalkley, Protoplasma, 24: 607, 1935). Text p. 274.

2. Influence of Substratum.—Place an amoeba in a weak gelatin solution to eliminate contact with substratum. Is movement impossible? (cf. Haycroft and Carlier, Proc. Roy. Soc. B, 15: 220, 1888; also D'Arcy Thompson, "Growth and Form," p. 211).

3. Mechanical Stimulus.—Touch the amoeba with a fine glass point. Note any change in direction of locomotion, in streaming, or contact with substratum. The reaction is slow. Cf. Seifriz, "Protoplasm," p. 57.

4. Chemical Stimulation.—Bring the tip of a fine capillary containing various solutions against the side of an amoeba. Try NaCl, Sugar, HCl. Note reaction, if any. Allow a drop of methyl violet to diffuse toward an amoeba. Result? Cf. Jennings, "Behavior of the Lower Organisms," p. 187, 1904.

5. Electrical Stimulation.—Observe an amoeba when electric shocks are sent through the water on a slide by means of 2 strips of tin foil (making good contact with the water on each side), and connected to an inductorium (2 dry cells). Begin with very weak shocks and note the effect on the protoplasm of the amoeba. Compare with the response of Tradescantia protoplasm described by Kühne. Cf. Bayliss, pp. 21–22. Note the form of minimal area. Text p. 76.

6. Contraction of Plant Protoplasm.—Repeat the experiment using filaments of **Spirogyra** placed lengthwise in water on a slide. Sketch a cell showing spirals contracted by the protoplasmic threads (cf. Scarth and Lloyd, p. 33).

7. Migration of Granules in Chromatophores.—Remove a few scales from Fundulus. Rinse in tap water and distilled water. Place in 0.1 M NaCl. Observe the chromatophores under the microscope. Na produces expansion (dark color of animal). Discard any scales showing "contraction" (clumped granules). Now place the scales in 0.2 M KCl after allowing NaCl to drain off. Observe K contraction followed by slow expansion. If time permits try 0.2 M $CaCl_2$ (cf. Ponder, "Essentials of General Physiology," pp. 149–150).

VIII. CILIARY MOVEMENT

1. Effects of Ions on Ciliary Action.—Obtain small fragments of gill (not mantle) from a live clam. Keep in sea water. Bits of gill are to be placed in labelled watch glasses containing the solutions listed below. Wash the tissue in the solution to be used to remove sea water. Observe under microscope and determine the duration of the ciliary beat. Keep microscope free from solutions. Text p. 283.

(a) Pure solutions of the chief cations found in sea water (and in blood) in M/2 concentration (isotonic with sea water). NaCl; KCl; $MgCl_2$; $CaCl_2$; sea water control.

(b) Combinations of two cations

25 vols M/2 NaCl plus 1 vol. M/2 KCl
25 vols M/2 NaCl plus 1 vol. M/2 $CaCl_2$
25 vols M/2 NaCl plus 1 vol. M/2 $MgCl_2$

(c) Combinations of 3 or 4 cations

25 vols NaCl plus 1 vol KCl plus 1 vol. $CaCl_2$
25 vols NaCl plus 1 vol. $CaCl_2$ plus 1 vol. $MgCl_2$
25 vols NaCl plus 1 vol. KCl plus 1 vol. $MgCl_2$
25 vols NaCl plus 1 vol. $CaCl_2$ plus 1 vol. KCl plus 1 vol. $MgCl_2$

Determine the duration of the ciliary movement in each case. Solutions of three salts and sea water controls should be observed next day. Cover watch glasses to prevent evaporation. Is KCl, $CaCl_2$, of $MgCl_2$ the most effective in antagonizing the toxic effect of NaCl? The effects are essentially those of ions on colloidal systems. Text p. 282.

If possible, distinguish between frontal (at distal ends of filaments) and lateral cilia. Cilia reverse their direction too rapidly to be followed by the human eye but the form of the moving cilium may be demonstrated with the stroboscope. Cf. Jennison and Bunker, Jour., Cell Comp. Physiol., 5:189, 1935. The data from all groups may be averaged. Consult Gray's book, "Ciliary Movement," Chap. V. Compare data

with salt effects on wheat seedlings (p. 14) muscle (p. 58) and heart (p. 88).

2. Schäfer's Hypothesis of Ciliary Movement. (a) **Cilium.**—Cut a narrow strip of paper (2 ins. in length) parallel to the shorter edge of the sheets provided (stationary or letter head bond). Wet one side with water and observe change of shape.

(b) **Flagellum.**—Cut a long narrow strip along a diagonal of a sheet of paper provided. Wet one side. Observe and sketch the form of the strip. Text p. 282.

According to Schäfer units in the vibratile structure of a cilium expand under hydrostatic pressure. Cf. Gray, p. 55. Text p. 286.

3. Cilia in the Frog.—A pithed frog is fastened dorsum down on a frog board. Remove viscera except oesophagus and stomach. Cut through lower jaw in mid line and continue the cut to the stomach. Pin back the sides of lower jaw and oesophagus to form a flat surface on a level with the roof of the mouth. Irrigate with Ringer solution. Avoid excess fluid. Text p. 285.

According to Lucas, Proc. Soc. Exp. Biol. Med. 30: 501, 1933, the excised pharyngeal epithelium of the frog gives rhythmic ciliary movement but **in vivo** these cilia are at rest and move only when excited.

(a) Place a bit of cork on exposed membrane. Determine direction of movement. Try heavier pieces of cork. Tip the board so the cork is carried up an incline.

(b) Determine time in seconds for movement of cork through one inch. Determine again after warming with saline at 30°C.

(c) Blow fumes of ether on preparation (use saturated filter paper). After a few seconds determine the speed again.

4. Effect of a Series of Alcohols on Ciliary Motion (Traube's Rule).—The following alcohols will be tested as narcotics on cilia:

Alcohol	Molecular Weight	Weight of 1 cc.
Methyl	32	0.80 gr.
Ethyl	46	0.79
Propyl	60	0.80
Butyl	74	0.78
Amyl	88	0.82

(a) Remove fragments of gill (not mantle) from a living clam and place in sea water. Place a bit of gill in a watch glass containing exactly 5 cc. of sea water on the microscope stage. With a calibrated medicine dropper add drops of the given alcohol at two min. intervals. Stir. Determine number of drops required to produce narcosis (half the cilia stopped). Return to sea water and determine time for recovery. CALI-

BRATE THE DROPPER USED FOR EACH ALCOHOL. i.e., determine the number of drops to make 1 cc. (in a 5 or 10 cc. graduate). Record this calibration on label attached to dropper. ALL DROPPERS MUST BE HELD EXACTLY VERTICAL.

(b) Calculate the molarity of the narcotising solution of each alcohol and record in a table. Text p. 58.

Sample Calculation.—Given 15 cc. sea-water, to which have been added 20 drops (from a medicine dropper) of ethyl alcohol. Suppose 30 drops of ethyl alcohol from this dropper are required to make a volume of 1 cc. and that 1 cc. of ethyl alcohol weighs 0.79 grams. Find the molarity of the ethyl alcohol in the mixture.

The 20 drops of ethyl alcohol added are equivalent to $^{20}/_{30}$ of 1 cc. and the total volume of the mixture is therefore 15 cc. + $^{20}/_{30}$ cc. or 15.67 cc. The 20 drops of alcohol weigh $^{20}/_{30}$ of 0.79 grams, or 0.527 grams. The mixture of 15.67 cc. therefore contains 0.527 grams of alcohol.

A 1 M solution of ethyl alcohol contains 46 grams of ethyl alcohol in 1 liter of solution. The number of grams x of ethyl alcohol that would be contained in 1 liter of our mixture is found from the proportion

$$\frac{0.527}{15.67} = \frac{x}{1000}$$

Solving for x, we find it to be 33.63 grams.

The molecular weight of the alcohol is 46, and the number of moles y of alcohol in 1 liter of the mixture is then found from the proportion

$$\frac{1}{46} = \frac{y}{33.63}$$

Solving for y, we find it to be 0.73.

The molarity of the mixture is therefore 0.73 M.

The above experiment was taken from G. Saslow's, "Experiments in Elementary Physiology," 1930.

5. Modification of Ciliary Beat by Electrical Current.—(a) Place on a slide with Ringer's fluid a strip of ciliated epithelium from roof of frog's mouth. (Clam gill can be used in sea water.) Add carmine particles and observe under microscope. Stimulate with induction shocks from tin foil electrodes attached to slide by rubber bands at each side of preparation.

(b) Connect a rocking key to two dry cells. Place a drop of thick Paramecium culture in a watch glass on a dark background. Dip wires from the key on each side of watch glass. Change direction of poles. Observe white specks (Paramecia) collecting at one pole. Do they swim

to cathode? Observe with a reading glass. Cf. Loeb, "Forced Movements," p. 44. Text p. 331.

IX. MUSCULAR MOVEMENT AND NERVOUS ACTION

APPARATUS

Precautions.—Handle all equipment with great care. Keep metal parts free from salt solution (especially muscle levers). Avoid scratching drum when removing kymograph paper. Do not bend tips of electrodes. Smoke drums lightly. Students who have not received adequate training in physics should read "Physical Principles of Electricity and Magnetism" by R. W. Pohl (trans. by W. M. Deans) 1930.

1. Electrolysis.—Apply two wires connected to a dry cell to neutral litmus paper moistened with NaCl solution (or Ringer). Note reactions at anode (positive) and cathode (negative). Apply also to filter paper moistened with starch solution containing KI. Explain result.

2. Induction Coil.—Wires of two circuits are arranged in parallel coils—**primary** circuit (inducing) and **secondary** circuit. When current is made or broken in the primary circuit, or when its intensity is altered, a momentary induced current appears in the secondary circuit. An automatic interrupter is inserted in the primary circuit. Place the primary coil in circuit with a simple key and dry cell. Make a diagram of the instrument, showing its arrangement and connections. The left and centre binding posts are used for single shocks and the outer ones for the interrupter.

Attach electrode wires to the secondary coil and apply to "pole-finding paper." (Starch plus KI.) Arrange the primary circuit to provide single shocks. Close and open the key a number of times. Result? Repeat, but cross circuit the secondary coil each time before breaking. Result? Repeat, but cross circuit so as to eliminate the "make."

Separate the two coils by pushing out the secondary. Close the primary circuit and then place the electrodes on the tip of the tongue. Result? Now make and break several times. Result? Slide secondary coil nearer. What is effect on the intensity of the shocks? Is make "stronger" than break?

3. Pole Changer (Reversing Key, or Rocking Key).—Study its mechanism. Draw diagrams showing the connections required for its use (1) to reverse current, (2) as a double key without changing the wires, (3) as a single key. Observe insulated ends of wires (rubber bushing).

4. Non-polarizable Electrodes.—(Can be supported with plasticine or holder.) These are soaked in Ringer's solution, then filled with $ZnSO_4$ solution in which a Zn rod is dipped. $ZnSO_4$ must not be spilled on the outer surface of the electrodes. Describe the mechanism of current con-

duction when these are applied to a tissue. Place litmus paper moistened with Ringer across the electrodes. Wire zincs to a dry cell.

Does electrolysis occur? Wash out the $ZnSO_4$ solution and dry the Zn rods. Place the electrodes in Ringer solution until needed again. Compare other types of non-polarizable electrodes (cf. Mitchell, "General Physiology," 1932, p. 21).

5. Kymograph.—Examine muscle levers, writing levers, signal magnets, femur clamp, tuning fork (electrical). These instruments require extremely delicate manipulation. The writing levers, mounted on a stand on the right, must be tangential to the drum. In every experiment set up the apparatus before making the dissection.

MUSCLE CONTRACTION

Striated muscle is characterized histologically by a double helicoid or spiral which appears as striations when viewed from the side (cf. Tiegs, Proc. Roy. Soc. B, 116: 38, 1934). **Examine and draw** a common test tube brush as a good example of a double helicoid. Text p. 291.

Nerve Muscle Preparation.—Destroy brain and spinal cord of a frog by pithing with a nail (as demonstrated). Remove skin from the frog (except from the head); skin secretions injure the muscles and nerves (cf. Szabuniewicz, Pflügers Arch., 223: 744, 1930). Note on dorsal thigh surface a longitudinal depression between vastus externus and semimembranosus muscles. The sciatic nerve lies in this groove, with blood vessels. Lift it **gently**, and isolate it as far as the knee. Then separate the thigh muscles with forceps and follow the nerve toward the spinal cord. **Avoid putting traction on the nerve.** Tie a moistened silk thread on the proximal end of nerve. Cut its roots as near the cord as possible. Cut the femur through the middle and remove the thick muscles. Tie a thread on Achilles' tendon. Cut the tendon of Achilles below the ankle. Lift the gastrocnemius muscle from the leg and cut through bone just below the knee. The preparation must be moistened constantly with Ringer.

Fix cut end of femur in a femur clamp and lay the nerve on a glass slide or across the electrodes.

1. Efficiency of Make and Break Shocks.—With 2 dry cells in circuit use inductorium for single shocks (left and center terminals). Attach the muscle to muscle lever for writing on kymograph. Arrange with great care to give muscle full mechanical advantage. Mark a base line. On a stationary drum record the height of contraction elicited by make (closing) and break (opening) shocks. Carefully label the records M or B. Move the drum 5 mm. between records. If no contraction occurs indicate by a short line **beneath** the base line. The make shocks are

weakened by Faraday's extra current (cf. Halliburton and McDowall, "Handbook of Physiol.," 19th Ed., p. 27, 1930).

2. Strength of Stimulus and Height of Contraction.—Make a base line. On a stationary drum record at 5 mm. intervals the height of contraction to stimuli of gradually increasing strength (start with the coils well separated). Use one dry cell. More fibres contract on strong stimulation. Define the terms minimal and maximal stimulus (cf. Howell, "Textbook of Physiology," 12th Ed., Fig. 9). Text p. 298.

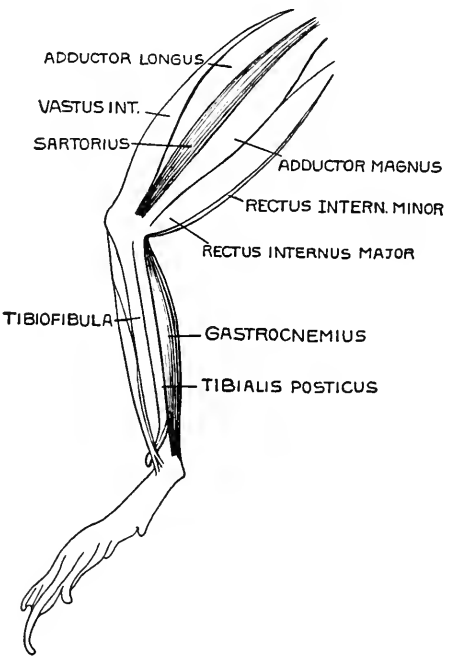

Fig. 3.—Ventral aspect of frog's leg to show position of the sartorius muscle.

3. Non-electrical Stimuli. (a) **Chemical.**—On a stationary drum record the contraction produced by placing crystals of NaCl on the nerve resting on a glass slide. Wash off with Ringer.

(b) **Mechanical.**—Obtain a contraction by pinching the nerve with forceps.

(c) **Thermal.**—Touch the nerve with a heated glass rod and record contraction. Label all records carefully before fixing in shellac.

4. Irritability and Water Metabolism. (a) **Drying.**—Set up a nerve-muscle preparation for obtaining records and allow the nerve to dry. Stimulate occasionally to test irritability. As the experiment may last two hours, proceed with the next experiments. Now apply Ringer's solution to the nerve with a fine brush. Does it recover? Label records.

(b) **Action of Distilled Water.**—Remove a sartorius muscle (Fig. 3) from a frog as follows. Seize the sartorius tendon (ventral aspect of leg) at the tibial insertion (knee). Lift the free end and separate the muscle up to the origin by snipping the fascia on each side. Sever at origin and suspend half-immersed in distilled water. Observe for 10 mins: movements; change in volume or color. Cf. Baldwin, "Comp. Biochem.," 1937, p. 17.

(c) **Hypertonic Saline.**—Immerse another sartorius muscle in 20% NaCl and observe for ten minutes. Note any effects.

5. Galvani's Experiment.—Prepare a sciatic gastrocnemius preparation for obtaining records (stationary or moving drum). Hold in contact a short zinc strip and a short bent copper wire. Touch the free ends of the metals to the nerve. Explain result. If possible obtain a record. Cf. Fulton and Cushing, Annals of Science, 1: 239, 1936. Text p. 295.

6. Electromotive Phenomena in Muscle (Rheoscopic Frog).—Make two nerve-muscle preparations designated A and B. Lay nerve of A lengthwise along muscle of B. Stimulate the nerve of B as far as possible from the muscle, with a submaximal induction shock. Result? Stimulate with interrupted current. Prove that the "secondary contraction" is not due to escape of current. The nerve may be placed on a vigorously beating heart. Cf. Lillie, Am. Journ. Physiol., 34: 414, 1914; Lillie, "Protoplasmic Action," 1932, p. 392.

7. The Curve of Muscle Contraction.—(Nerve not required for this experiment.)

Mount a gastrocnemius preparation without the nerve on the muscle lever and load it with a ten-gram weight. Connect the ends of the muscle to the poles of the secondary coil of an inductorium by fine copper wires. Put a signal magnet and a simple key in the primary circuit. Arrange a writing point on a tuning fork to record hundredths of a second. Bring the three writing points into the same vertical line, and as close together as possible. All levers should be tangents to the drum. It will be most convenient to have the muscle lever above and the tuning fork and signal below. Set tuning fork in vibration. Avoid superimposing time records.

Spin the drum rapidly by hand, and record the curve obtained from a maximal shock. Stop the drum, remove the tuning fork, but carefully avoid disturbance of the relative position of the other two points. The tuning fork must be allowed to mark only during the revolution in which the curve is actually made. Turn the drum cautiously until the signal is exactly at the point where the shock was given and mark the corresponding position of the muscle lever (simultaneous coordinates). Calculate in fractions of a second (1) the latent period, (2) the period of shortening, (3) the period of relaxation. Make several determinations. The nature of the latent period is described by Snyder, Am. J. Physiol., 115: 441, 1936. Text p. 294.

8. Summation of Stimuli.—Send in two shocks in rapid succession by making and quickly breaking, so as to obtain a curve showing what happens when a muscle is stimulated at the height of contraction. If you fail in obtaining the correct time interval between shocks the first time, try again. No time record is required. Text p. 295.

9. Incomplete Tetanus.—Repeat, making and breaking by hand in rapid succession. Summation of several shocks should be obtained, giving an incomplete tetanus. No tuning fork.

10. Complete Tetanus.—Describe a curve of contraction with tetanizing current, i.e., with interrupted faradic or induced current. Do not stimulate for longer than 3 seconds. No tuning fork. Cf. Nicolai, Pflüger's Arch. 237: 399, 1936. Text p. 295.

11. Isometric Contraction.—Record twitch (weak shock) with the isometric lever (supplied by instructor). Superimpose the curve on a calibration line made when a ten gram weight is placed at the point of attachment on the lever. No tuning fork. Cf. Wiggers, "Physiology," 1st Ed., p. 8, 1934.

12. Fatigue Curve.—With very slow speed of drum, fatigue the muscle by prolonged tetanus. Note the gradual relaxation in spite of continued stimulation. When completely fatigued, allow to rest. Wash with Ringer solution. Then take curves of single twitches with maximal break shocks. Compare with the curves from the fresh muscle. No tuning fork. Cf. Simonson, Ergebnisse Physiol., 37: 299, 1935.

13. Influence of Circulation on Fatigue.—Anaesthetize a frog with urethane (1 cc. of 10% sol. to 50 grams of frog injected into dorsal lymph sac), and place dorsum down on frog board. Make small incisions in the skin of the ankle and free the Achilles' tendons. Avoid hemorrhage. Tie threads to tendons and attach one to a muscle lever weighted with 20 grams. Insert fine wire electrodes through skin into gastrocnemius by means of needles threaded on the fine wire. Pass a linen ligature about the thigh and tie off circulation. For connection to lever, pass thread over a pulley consisting of a spool rotating on a rod. Stimulate at once with a maximal tetanizing current to obtain a fatigue curve. Now obtain a curve from the other leg. Study the effect of rest in each case.

14. Fatigue of Human Muscle.—Clamp the L-shaped ergograph to the table. Allow the adjustable rod to rest on the index finger and record a fatigue curve on the kymograph.

15. Artificial Tetanus of Human Muscle.—With hand in ergograph as above place on the forearm a flat metal electrode covered with cotton wet with salt solution (indifferent electrode). With a metal rod electrode similarly covered with moist cotton touch the skin over the abductor of the index finger near the angle between the first and second metacarpals.

Stimulate with a tetanizing current for a few seconds and compare the resulting kymograph curve with that of a short voluntary tetanus.

16. Determination of Motor Points.—Let the left arm rest on the indifferent electrode (copper plate covered with cotton soaked in Ringer) and explore the surface of the left forearm with the stimulating electrode (copper rod with tip covered with cotton soaked in Ringer) for motor points (for long flexor of thumb—2 points for flexions of digits—for ulna nerve at elbow—deltoid muscle on shoulder, etc.) Mark skin with ink. Make a chart. Use tetanizing current. Cf. Howell, 12th Ed., Fig. 35; Tabulae Biologicae, 2, Plates XIV–XV, 1925.

17. Polar Stimulation of Human Nerves.—Connect a pole changer (rocking key) to six cells in series. The subject holds the indifferent electrode, while the observer presses the stimulating electrode over the motor point of the ulnar nerve at the elbow. Make the stimulating electrode the cathode and close and open circuit. Reverse current by means of current by adding dry cells repeating procedure each time. How many cells are required in each of the four cases? This method is used in the diagnosis of nerve injury (cf. Howell, 12th Ed., p. 106). The normal sequence is cathode closing, anode closing, anode opening, cathode opening (order of efficiency).

18. Polar Changes of Irritability (Catelectrotonus).—Make a sciatic-gastrocnemius preparation of frog with a long nerve (as far as spinal cord). Connect non-polarizable boot electrodes to end posts of a pole-changer with cross wires in circuit. Attach middle posts to 2 dry cells. Do not spill $ZnSO_4$ on outside of electrodes. Place the nerve across the boot electrodes. Arrange ordinary pt. electrodes to stimulate the nerve between the boot electrodes and the muscle. One boot electrode should be near the pt. electrodes. With drum rotating slowly stimulate the nerve through the pt. electrodes with the **weakest interrupted current maintaining a slight tetanus** (one dry cell). Now send for a moment through the nerve an ascending galvanic current (with the anode of the boot electrodes near the pt. electrodes). Break first the galvanic current; then a moment later the tetanizing current. If necessary use a stronger galvanic current. Repeat with a descending current (boot cathode next the pt. electrodes). Do both poles of a galvanic current have the same effect on irritability? Cf. Howell, 12th Ed., p. 101. Text p. 348.

19. The Rate of Transmission of a Nerve Impulse.—Make a sciatic-gastrocnemius preparation but split the vertebral column and retain the entire length of the nerve. (Use a bull frog if available.) Connect two pairs of electrodes to a rocking key. Lay the nerve on the two electrodes, one near the muscle, and the other close to the piece of vertebral column. Take a simple twitch (with signal key and tuning fork) when the rocking key is arranged to stimulate through the far electrode.

Spin drum very rapidly. **Only the initial rise of the curve is required.** Take another twitch with the key rocked over to stimulate through the near electrode. Measure the distance between the two electrodes and record this distance on kymograph. Observe the time difference between the two latent periods. Calculate the rate of transmission of the impulse in metres per second (cf. Howell, 12th Ed., p. 117). Estimate the chronaxie of this nerve. The impulse travels one cm. in one chronaxie. Cf. Lapicque, L'Excitabilité, 1926, p. 356. Discuss the speed of impulses in various nerve fibres. The rapidity of the various fibres in a nerve depends on their irritability and diameter. Helmholtz first measured the rate of a nerve impulse (Arch. Physiol., 1850, p. 71). Cf. Mitchell, 1932, Fig. 11, p. 23. Text p. 344.

20. Augmentation of Muscular Chronaxie.—Attach a sciatic-gastrocnemius preparation to the myograph lever in the usual way. Apply one pair of electrodes to the nerve and another pair, composed of fine wire, to the muscle. The electrodes are connected by a rocking key. Arrange the switch to excite through the nerve and stimulate with a simple key once per second with the drum moving slowly and the muscle lifting thirty grams at each contraction. Record the contractions until the muscle ceases to raise the weight. Then rock over the key to stimulate the muscle directly and record the contractions again until fatigue is complete. Fatigue increases the muscle chronaxie and conduction from the nerve is blocked. (Heterochronism.) Cf. Lapicque, "L'Excitabilité," p. 278. For recent views on chronaxie cf. Davis and Forbes, Physiol. Rev. 16: 407, 1936. Text p. 358.

21. Contraction of Hyoglossal Muscle (Red Fibres).—Remove whole lower jar and hyoid cartilage with tongue attached from frog used for gastrocnemius studies. Tie a thread to the tip of the tongue and fix the hyoid with pin to a slice of cork held in a femur clamp. The tongue may pull vertically on the lever (not weighted). Stick electrodes into muscle just in front of hyoid. Take a simple twitch with signal key and tuning fork. Compare latent period and duration of contraction with those obtained with gastrocnemius (white fibres) (cf. Ranvier, Comp. rend. Acad. Sci. Paris, 77: 1030, 1873; Hinsey, Physiol. Rev., 14, 565, 1934). Text p. 318.

22. Contraction of Smooth Muscle.—Make two transverse cuts across a frog's stomach to obtain a ring of smooth muscle about 5 mm. wide. Place in Ringer until relaxation occurs. Fasten a fine wire to inductorium and bend up at end. Clamp at bend and fasten the end through the ring of muscle. Fasten a straight fine wire to the other part of the inductorium, bend last 2 ins. down and fasten through ring of muscle. Attach elbow of upper wire with thread to a heart lever. Stimulate with a strong shock or tetanizing current. Compare curve with those from

skeletal muscle and loops of earthworm (cf. Bayliss, pp. 436–37). Earth worms may be used for this experiment. Text p. 289.

23. Microscopic Study of All-or-None Effect in Skeletal Muscle.—Carefully remove a sartorius muscle. Place in Petri dish. Fasten down one end firmly with a pin passing through a cork attached to rim of dish (see demonstration). Similarly fasten down the other end with a needle **covered with wax except at extreme tip.** Determine cathodal post of inductorium for break shocks (eliminate makes) using starch-KI paper. Wire stimulating needle as cathode. Shake a bottle of charcoal and blow escaping fine dust on muscle. Cover with Ringer into which dip a wire (anode) from other post of inductorium. Observe muscle through microscope and stimulate with break shocks with a **mercury key** in primary circuit. Find particles that move lengthwise of the muscle. Starting with a just threshold break move secondary coil closer, a mm. at a time, and observe relation between strength of stimulus and movement of particles. Compare results when a single fibre is excited and when many fibres are in action. Are effects compatible with the All-or-None Law? (cf. Pratt and Eisenberger, Am. J. Physiol., 49: 1, 1919). For a review of the All-or-None Law, cf. Rosenbleuth, Quart. Rev. Biol., 10: 334, 1935; Brown and Sichel, Jl. Cell. Comp. Physiol., 8: 315, 1936. Text p. 299.

24. Isolation of Impulse Path.—Wire a needle to one post of inductorium arranged for minimal tetanizing currents. Skin the legs of a pithed frog and expose the sacral nerve plexus. With a copper wire "ground" the head (to water or gas pipes) and touch the sacral nerves here and there with the needle electrode and observe effect on sartorius or other muscles. Do all fibres contract?

25. Does a Nerve Fibre Conduct in Both Directions?—Carefully sever a gracilis muscle at its insertion and turn to reveal inner surface. Note where the nerve and blood vessels divide going to each side of the muscle. Cut muscle in half between the nerve branches. Avoid injury to nerve. Stimulate one half. Does the other half contract?

26. Reciprocal Inhibition in Crustacean Muscle (Fig. 4).—Remove a leg from a lobster or crab (or claw of crayfish). Note a sequence of flexion and extension produced by stimulus of cutting off the limb. With a probe make holes in the shell on the leg for electrodes (near proximal end). Clamp limb securely so that only the terminal segment (dactylus) is moveable. Plasticine may be used. Attach the dactylus with thread to a heart lever. Irrigate with sea water or Crustacean Ringer solution. Make a base line to distinguish between extension and flexion on the record. Starting with a very weak tetanizing current determine the effect of gradually increasing the strength of stimulus (continuous stimulation). The dactylus must be placed in a half way position so that flexion or extension may be recorded. These preparations are very

perishable. Try several until satisfactory results are obtained (cf. Bayliss, p. 404, or Barnes, Sell and Spofford, Zeit. verg. Physiol., 18: 282, 1932). Text p. 300.

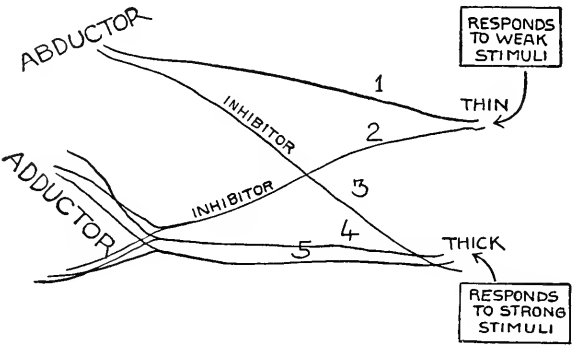

Fig. 4.—Nerve fibres to adductor and abductor in crustacean claw (cf. Barnes, Am. J. Physiol., 107: 447, 1934).

27. Effect of Temperature on Muscular Contraction.—Make a gastrocnemius preparation of the frog without the nerve. Bind the femur to the L-shaped rod of the "muscle warmer" (supplied by instructor) with uninsulated fine wire. Attach the tendon of Achilles to the muscle lever by fine wire, the free end of which is attached to the binding post of the lever to which one post of the inductorium is wired. Wire other post of inductorium to L-shaped rod of warmer. Place 1 cc. of Ringer in the glass cylinder and fix it in place. Arrange to stimulate muscle with a maximum induction shock. Weight with 10 grams. Insert thermometer in muscle warmer. **With every change of temperature wait 5 mins. for thermal adjustment.** Immerse muscle warmer in a glass jar of water, the temperature of which is adjusted with hot water or ice. Record contractions on rapidly moving drum with electrical time record. Start at room temperature and proceed to 0° at 2° intervals, then raise the temperature taking records at 2° intervals until **heat rigor** sets in. Note height and duration of contraction at each temperature (cf. Howell, 12th Ed., p. 31). If necessary, time may be saved by taking the records on a slow drum without tuning fork.

28. Effect of Curare.—Pith the brain but NOT the cord of a frog. Slit the skin on the dorsal side of the left thigh. Expose the sciatic nerve. Avoid injury to blood vessels. Insert a moist thread ligature **under the nerve** and tie tightly around the thigh to stop circulation. Cover exposed nerve with filter paper soaked in Ringer solution. Inject 3 or 4 drops of curare solution into the dorsal lymph sac. The body will become limp in about 10 mins. Suspend by a hook in the jaw. When tactile stimulation of the right leg fails **expose both sciatic nerves up to the vertebral**

column. Stimulate the right sciatic near the column with a submaximal stimulus. Do the muscles contract? Try the left sciatic. Remove skin over right gastrocnemius and apply electrodes directly to muscle. Explain. Discuss the theories of curarization of Bernard, Lapicque and Bancroft. Cf. Starling, "Physiology," 7th Ed., 1936, p. 126. Text p. 359.

29. Salt Effects on Muscle.—Carefully remove curarized small muscles (sartorius, biceps, tibials, etc.) from the leg of a frog. Place in the following solutions. Change each solution 2 or 3 times to remove foreign matter.

(a) M/4 sugar solution
(b) 4 vols. m/4 sugar + 1 vol. m/8 NaCl
(c) M/8 NaCl. When irritability is lost add drops of $CaCl_2$
(d) M/8 NaBr. When irritability is lost add drops of $CaCl_2$
(e) 24 vols. m/8 NaCl + 1 vol. m/8 $CaCl_2$
(f) M/8 KCl
(g) M/12 $CaCl_2$ (why not m/8?)
(h) M/8 sea water (sea water equivalent to 0.52m NaCl)
(i) Controls in Ringer's solution, including one in ice box for several days.

Note any immediate effects. Test irritability with single induction shocks at 10 min. intervals. Note any contraction, permanent shortening, twitching. Which solutions preserve irritability longest? After irritability is lost, test recovery in Ringer solution (cf. Höber, "Physikaliche Chemie der Zelle und der Gewebe," 6 Auf., p. 641). The twitching in sodium salts was first observed by Biedermann (cf. Chao, Am. J. Physiol., 109: 550, 1934). Text p. 291.

30. Effect of Veratrin.—Inject 0.5 cc. of veratrin solution into the dorsal lymph sac of a frog with brain pithed. Note any effects on reflexes and on general activity. Make a gastrocnemius sciatic preparation and record a twitch (break shock). Note double peak and prolonged relaxation. Stimulate several times. Does the effect wear off? Test the effect of rest (cf. Howell, 12th Ed., p. 33; Bayliss, p. 734). Text p. 359.

X. ELEMENTARY PHYSIOLOGY OF THE CENTRAL NERVOUS SYSTEM

1. Action of Strychnine.—Pith the brain (not cord) of a frog and inject a few drops of 0.5% strychnine sulphate in the dorsal lymph sac. Suspend the frog by a bent wire hook through the jaw. After a few minutes apply a weak tactile stimulus to any part of the body. When contraction ceases, touch at other points. Describe result. What effect has the drug on graded resistances in the C.N.S.? Text p. 359.

2. Delayed Conduction at Synapse.—Make a gastrocnemius preparation retaining both sciatic nerves including the connecting bit of vertebral

column. Arrange 2 pairs of electrodes (rocking key) to stimulate the nerves on each side of the fragment of chord. Record a twitch in each case with rapid drum and tuning fork. Compare latent periods. For properties of the synapse see Bayliss, p. 141; Eccles, Er. Physiol., 38: 339, 1936.

3. Summation in a Reflex.—Pith the brain **but do not pith the spinal cord** of a frog and suspend the animal by a hook in the jaw. Tie two fine copper wires 1 cm. apart around the toes of the left foot. Connect wires with secondary coil of inductorium. Connect primary with single cell and simple key. Can reflex action result from summation of **weak** make and break shocks? Try a tetanizing current of subliminal stimuli (cf. Bayliss, p. 489).

4. Inhibition of a Reflex.—Immerse toes of right leg in weak acetic acid solution and note time for the reflex. Wash off the acid. Now stimulate the left foot with weak tetanizing current as the right is again immersed in the acid. Result? (Bayliss, p. 410.)

5. Chemical Stimulation by Ions.—Dip the frog's foot into 2 M LiCl and time the latent period of reaction. Compare with 2 M KCl. The stimulating efficiency of ions is a function of their mobility determined by molecular weight and their shell of water molecules. Li, although of small mol. wt. (7), moves more slowly than K (39) because of hydration. Li carries 150 water molecules, K only 20. Hopkins (J. Exp. Zool., 61: 13, 1932) discusses ionic mobility and stimulation (cf. Maass and Steacie, Physical Chemistry, 1931, p. 220, for ionic movement and hydration). Text p. 34.

6. Irradiation of Reflexes.—Gradually increase the strength of a very weak tetanizing current and note effect on the reflex movement of the various limbs. (Crossed reflexes.)

7. Reflex Tonus.—Note position of the legs of the suspended frog. Slit abdomen and cut roots of all nerves going to right leg where they leave the spinal cord. Again suspend the frog and note position of the legs (cf. von Buddenbrock, "Grundriss der vergl. Physiol.," p. 186).

CENTRAL NERVOUS SYSTEM OF CRAYFISH

Directions from "Laboratory Outlines in Comparative Physiology," by C. G. Rogers, McGraw-Hill, 1929.

8. Functions of Circumoesophageal Commissures.—Carefully expose the circumoesophageal commissure on the right side of a crayfish (Fig. 5). Insert probe in mouth and dissect from one side. Stimulate with a weak tetanizing current and observe effect on body and limbs. Note effect on voluntary movement of appendages. Note extension with strong currents (reverse of peripheral nerve stimulation see p. 54). Cut left circumoesophageal commissure. Does the animal now make spontaneous

locomotor movements? **Compare with an intact animal.** Stimulate with a needle. Note degree of flexion of appendages.

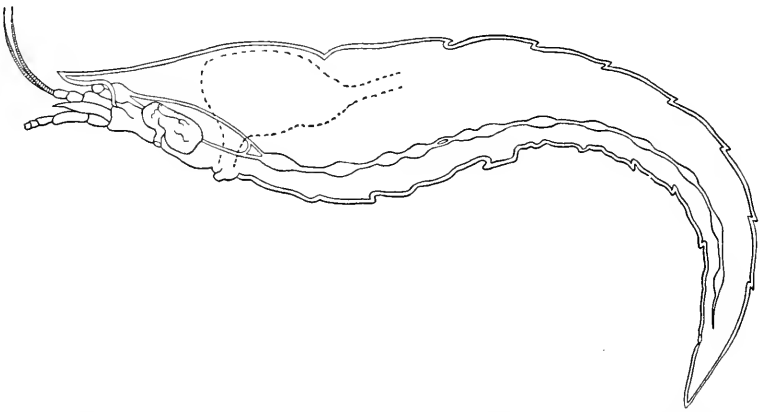

Fig. 5.—Diagram of the nerve cord of the crayfish. Note position of circumoesophageal connectives in relation to green gland (stippled) and alimentary tract (dotted lines).

9. Functions of Ventral Nerve Cord.—Cut the commissure between the suboesophageal and the first thoracic ganglia. Compare the behavior of animal in all points with that prior to the operation. Cut across the ventral nerve cord between the thorax and abdomen. Any voluntary control of appendages? Can individual appendages give reflex movements? Consult Roger's "Comparative Physiology," p. 525.

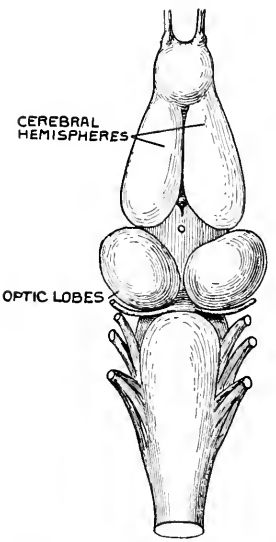

Fig. 6.—Dorsal aspect of frog's brain.

CEREBRUM OF FROG

10. Effects of Decerebration on the Frog.—(The following operation should be performed very carefully with well sharpened sterilized instruments.) Anesthetize slightly with ether a male frog (just ceases to show equilibrium reflexes). If effect of ether wears off replace in ether jar.

The cerebral hemispheres extend back to a line connecting front margins of tympanic membranes. Cut skin along this line over top of skull. From this cross-cut make median incision forward nearly to nostrils. At intervals wash with antiseptic Ringer's solution ($HgCl_2$ 1:2,000). Lay back the flaps. With scissor points separated to either side of top of skull, immediately in front of transverse incision, cautiously bring points together by cutting barely through bone. Insert the sharp blade

forward and at one side under the bony covering of cerebrum and snip the bone. Repeat on other side. Raise the bone with small forceps and carefully cut forward, alternately on one side and the other, until cerebrum is exposed. Sever connections between optic lobes and cerebrum and remove it. Sew together flaps of skin with silk thread moistened with antiseptic Ringer.

Note posture. Keep animal 1 hour in moist container and then compare with normal and spinal frog (whole brain pithed) as follows: (a) Record differences in **Posture, Locomotion** (Hopping and Swimming) and **Respiration.** (b) **Vision.** Compare eyelid movements. Stimulate frog to jump toward an obstacle 6 cm. distant in path of light beam. (c) **Equilibration.** Turn frog on its back. Compare reactions. Slowly tilt support of frog in normal position. (d) **Croak reflex.** Hold frog immediately behind front limbs. Apply temporary light pressure. (e) **Nystagmus.** Place the animal on rotating surface (phonograph or cardboard mounted on kymograph shaft). Note head movements.

If the operation is successful the frog should be kept for several days (in moist chamber) and tested again. Label the jar with your name and the date. For effects of decerebration on the frog cf. Mitchell, 1932, p. 145.

XI. PERMEABILITY AND OSMOTIC PRESSURE OF LIVING CELLS

The irritability of living cells as seen in the muscle and nerve experiments (above) is largely determined by the permeability of the plasma membrane which will be studied in the following experiments.

1. Membrane of Oxide on Metal.—Place a large globule of clean **Hg** into a watch glass of distilled water and another of the same size into a watch glass of 10% hydrogen peroxide. Stir each with a blunt point to separate into smaller drops. Note differences. With the blunt point gather the drops together again. Explain isolation of drops in H_2O_2. Gently tip the watch glass containing a large globule of Hg in H_2O_2. Observe oxide membrane on upper side (Lillie, "Protoplasmic Action," 1932, p. 247).

2. Iron Wire Model of Plasma Membrane of Nerve Fibre.—An iron wire covered with a film of oxide in nitric acid transmits an electrical "action current" resembling the nerve impulse. Touch the end of the iron wire in the demonstration with a Zn rod (why?) and observe the propagated disturbance indicated by bubbles. The "nerve" must be allowed to rest during the refractory period (rebuilding of film) after each stimulation (cf. Ponder, "Gen. Physiol.," p. 269, or Lillie, "Protoplasmic Action," 1932, p. 254; Biol. Reviews, 11:181, 1936). Text p. 196.

3. Permeability Changes during Stimulation.—Place **Spirogyra** filaments on a slide with pond water stained with **acid fuchsin.** By means of **tin foil** electrodes, stimulate with a **weak** interrupted current and observe

color of cells under microscope (cf. Bayliss, p. 139). Remove filaments to unstained tap water to observe color. Usually the cells are injured showing increased permeability when dead. Text p. 367.

4. Permeability of a Liquid Layer as a Function of Solubility.—Very carefully place a layer of **chloroform** having **iodine** in solution beneath distilled water in a flask. Above the **water carefully place a layer of ether.** Let the flask stand undisturbed. Set up a similar experiment in another flask but use **1% KI** (in which **I is more soluble**) instead of distilled water. The solubility theory of permeability proposed by Liebig in 1849 is discussed by Stiles, "Permeability," 1924, p. 46.

5. Traube's Copper Ferrocyanide Membrane.—Mix equal volumes of **10%** $CuSO_4$ and **2M sugar** solution. Introduce a drop of the mixture beneath surface of **1% potassium ferrocyanide** solution in a small beaker. Allow to stand. Explain changes in form (cf. Bayliss, p. 112). Text p. 179.

6. Artificial Cells with Protein Membrane.—Shake five drops of chloroform in a small quantity of distilled water. On standing do droplets of chloroform reunite? Repeat using **egg white solution** and **chloroform.** Note difference in behavior of chloroform droplets. Pour into flat dish and allow to evaporate. Do interfaces between chloroform and egg white disappear? Shake 10% solution lecithin (in chloroform) with egg white solution. Allow chloroform to evaporate and stain contents of flat dish with neutral red; use weakest possible solution of neutral red to obtain a delicate tint which will show action of alkali on the cells; examine under microscope; draw. Lecithin absorbs water as chloroform evaporates; each cell is a watery solution of lecithin surrounded by a modified protein film. To a small portion of these cells add **N/500** NH_4OH, and **N/500 NaOH.** Note the time for color change (yellow) **in each case.** Ammonia is lipoid soluble. Cf. Bayliss, p. 136; contrast with living cells. Try the effect of saponin. Harvey (Science, vol. 36, p. 564, 1912) devised these cells as plasmogenic models of sea urchin eggs. For recent speculations concerning the nature of the plasma membrane cf. Danielli, Jour. Cell. Comp. Physiol., 7: 393, 1936. Text p. 175.

7. Changes in Permeability of Beet Cells.—Cut **slices of beet** and wash in tap water to remove contents of **injured cells.** Place controls in tap water. Place slices in (a) **0.31M (1.82%) NaCl** which is isotonic with the protoplasm. (b) **1%** $CaCl_2$; (c) **chloroform water.** Boil several slices in tap water and test with **Benedict's** reagent after adding a drop of concentrated **HCl.**

Benedict's Test for Sugar.—Heat to boiling about 5 cc. of Benedict's reagent in a test tube with a glass fragment to prevent bumping. Add about 8 drops of sugar solution and boil for two minutes. If more than 2 or 3 tenths per cent sugar is present, the solution will be filled with a

colloidal (greenish yellow or reddish) precipitate. With smaller amounts of sugar the ppt. will appear on cooling. (Do not hasten cooling by immersion in cold water). Cf. Bayliss, p. 126.

8. Permeability of Protozoa.—Stain **Paramecium** in pond water plus **a trace of neutral red.** Place a drop crowded with red Paramecia in **N/500 NaOH,** another in **N/500 NH_4OH.** Record time for color change and for death in each case. **Explain. Where** does **NaOH** produce its effect? **Keep microscope free from solutions.** Text pp. 246, 252.

9. Do Ions or Molecules Penetrate?—Stain **Spirogyra** in **neutral red;** place in **NaOH N/40; $Ba(OH)_2$ N/40;** and **NH_4OH N/40;** watch under microscope and record time for colour change in each case. As soon as change is noted in filament, return it to pond water. Does red return? Place cells stained with neutral red in NH_4OH until yellow; then remove to NaOH. Do they become red? Explain. Test permeability of cells killed by saturated chloroform water. Cf. Mitchell, 1932, p. 453. Text p. 251.

10. Plasmolysis of Spirogyra.—Use only **large healthy filaments** (preferably **S. nitida**). Compare cells in pond water and in 2M sugar. Note osmotic distortion in this concentrated solution.

Now place normal Spirogyra cells in

M/2	M/3
Cane sugar	**Cane sugar**
Urea	**Urea**
Alcohol	**Alcohol**

Do they **plasmolyse** in each case? Note with care if plasmolysis is permanent. Explain differences. Which substances penetrate most readily? Which solutions have the same osmotic pressure? Observe ppt. of tannin by urea. Young cells are more permeable according to Weber (cf. Hober, Ann. Rev. Biochem., 1: 14, 1932). Recovery from plasmolysis is a useful test of penetration (cf. Stiles, "Permeability," p. 173, 1924). Text pp. 10, 247.

11. Penetration of O_2.—Carefully remove sartorius muscles from frog. Stain each in **.1% methylene blue in Ringer.** Place on slides in Ringer. Cover each with firmly oppressed cover-slip (no air bubbles). Note color at edge. Gently steam one slide to kill muscle. Lift cover-slips and observe time for return of blue color in each. Is dead muscle more permeable to O_2? Cf. Harvey, J. Gen. Phys., 5: 215, 1922. Text p. 258.

12. Permeability of Erythrocytes.—Place a drop of frog's blood corpuscles in distilled water in a watch-glass and **examine quickly** under microscope. Place a drop of corpuscles in **strong NaCl solution.** Observe effects (laking and crenation). Try **.3M urea solution;** it causes haemolysis as if it were water. Dissolve the urea in **.15M NaCl;** effect? Explain. Consult Ponder's monograph "The Mammalian Red Cell," 1934. Please make sure that no solutions have touched microscope lens or stage. Text p. 12.

13. Rate of Diffusion of NH_4OH and NaOH.—Cover one end of each of two tubes with a **collodion** film tied tightly over the ends of the tubes. Paste a strip of mm. paper on each tube. Fill the tubes with 2% **agar-agar** colored by neutral red. Immerse the covered end of one tube in **N/100 NH_4OH** and of the other in **N/100 NaOH.** Measure the rate of diffusion. Is there any real difference? Compare results with Exps. 8, 10, and 11. Plot the curve for the distance of penetration as related to time. Text p. 2.

14. Paradoxical Penetration of CO_2 through Epidermis.—Make an "artificial cell" by stretching frog's skin over the end of a glass tube, holding it in place with a rubber band. Put in the tube an aqueous solution of phenol red made very slightly alkaline with $NaHCO_3$. Suspend the tube with the skin just below surface of **N/100 H_2SO_4** colored yellow with a drop of **phenol red.** Allow to stand 5 minutes. No change should occur. Now with **N/2 $NaHCO_3$** make the outside solution distinctly alkaline. The internal solution soon becomes acid. Why?

Prepare 3 such cells, with the skins inside out. Place in each **1 cc. of phenol red solution at pH 7.4.** Dip the first into **N/10 HCl + NaOH to pH 7.4,** the second to HCl → pH 7.4 with NH_4OH, the third into **N/10 $NaHCO_3$** + CO_2 at pH 7.4. Color external solutions with phenol red. Stir contents gently from time to time. Observe for half an hour (cf. Mitchell, 2nd Ed., p. 454, for discussion of permeability to CO_2; Gellhorn, "Das Permeabilitätsproblem," p. 233, 1929; Jacobs, Am. J. Physiol., 53: 457, 1920). Text p. 259.

15. Perfusion of Kidney.—These experiments take time and skill; several attempts are required to develop technique. Use bull frogs if possible.

(a) **Is the Kidney Permeable to Large Colloidal Particles?**—Prepare fine cannula by drawing out medicine dropper in flame. Attach to small burette with gum rubber tubing. Etherize frog. Fold back flap of skin on belly (which will be sewed in place later) to expose fascia and muscles. Observe abdominal vein in median line. Pass 2 ligatures under vein, about ½ cm. apart. Tie off lower ligature. Cut halfway through vein and insert cannula pointing towards head. Tie second ligature. The burette should contain 5% azoblau. Fluid must flow from the cannula slowly as inserted to insure absence of air bubbles. Allow 1–2 cc. of fluid to enter very slowly over period of 10 min. Remove cannula, tying second ligature tightly. Sew up flap of skin.

After frog has recovered, in from 1–6 hours, catheterize to obtain urine (insert steeply tapering curved glass tube into cloaca, withdraw and look for blue color). Examine the excised kidney microscopically.

(b) **Concentration of Phenal Red in Urine.**—Two aspirator bottles are required each connected with gum rubber tubing leading to a fine cannula. Bottle A containing oxygenated Ringer should be about 24 cm.

above level of frog. Bottle B, about 12 cm. above level of frog, contains about 0.0005% phenol red in oxygenated Ringer. **In Höber's perfusion method a dye dissolved in Ringer solution is introduced into the frog's kidney from the renal portal vein while the renal artery is supplied by Ringer.** (See Fig. 7.)

Pith brain and spinal cord of male frog. Tie string tightly about each leg, where it joins the body. Make incision on each side, carefully,

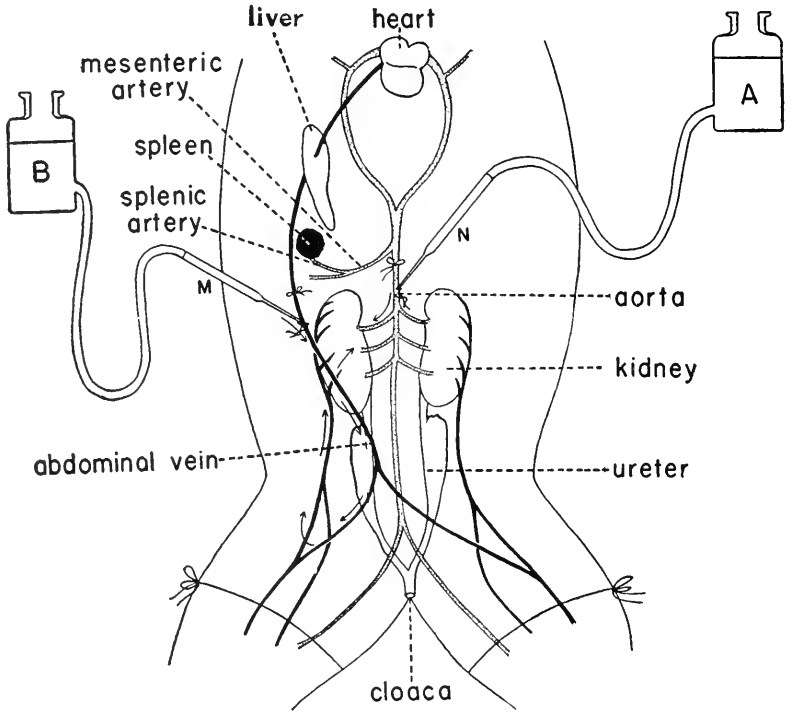

Fig. 7.—Diagram of circulation in frog's kidney with position of cannulae for perfusion (not drawn to scale). Sketch by Vance Tartar.

from lower part of abdomen to point ¾ in. above pectoral girdle. In doing this, cut through pectoral girdle with heavy scissors. The incision should be through the skin and muscles of the body wall. Join the two incisions by a cut across the base of the mouth.

Carefully lay back strip so delineated, cutting muscles as you do so. Stop before severing the vena abdominalis. This vein clings to the inner side of the strip and leads into the heart. Ligate the vein with thread, then sever from heart.

Remove the liver. Do not hesitate to sever the hepatic veins. Dissect out the intestinal tract. Be sure not to injure the spleen or the splenic artery, which runs to the abdominal aorta. Separate these from the rest

of the tissue, then remove the whole digestive tract by severing the esophagus and rectum.

Place frog on zinc pan, with plasticine supports arranged as in demonstration.

Holding spleen in forceps, trace splenic artery back to junction with abdominal aorta. Pass ligature under aorta. Delicately cut half way through aorta and insert cannula **N** from reservoir with colorless medium. Tie ligation. Insert other cannula **M** into vena abdominalis, likewise tying tip in place. CANNULAE SHOULD BE INSERTED WITH LIQUID FLOWING, to avoid air bubbles.

Cut away urinary bladder and find seminal vesicles and ureters. Under binocular microscope, incise vesicles and insert cannulae into the ureters. Tie in place. Watch preparation to see that flow of liquids is not blocked. In one-half hour note liquid issuing from ureters. Instead of cannulating ureters you may catheterize cloaca after an hour as in Ex. **15** (a). Text p. 246.

(c) **Simple Experiment on the Permeability of the Kidney.**—For the benefit of students who find experiments (a) and (b) too tedious the following simple experiment is included.

In an anesthetized frog make an incision in the ventral surface of the body wall from the pelvic region upwards through the pectoral girdle, cutting as few blood vessels as possible. If bleeding occurs cauterize the spot with a heated wire. Divide the skin and muscles of the abdominal wall on the right side by a transverse incision at the level of the kidney extending around the vertebral column. Divide the peritoneum on a line running parallel to the lateral border of the right kidney. Place the frog on a board or piece of heavy card board provided with a hole for microscopical observation. Pin the animal down to prevent reflex movements. Pin the kidney over the opening in the board. Avoid injury to the vascular system of the kidney. The region between the adrenal gland and the edge of the kidney is to be observed. The ventral surface of the kidney must be horizontal. Place a cover glass over the kidney to prevent evaporation. Keep the animal moist with cloth soaked in Ringer. Put the frog board on the stage of a microscope with light reflected through the kidney. Use low power. Locate the glomeruli. Prepare a hypodermic syringe with 0.5% indigo carmine. One student **slowly** injects half a cc. of dye into the heart while his partner watches through the microscope. If all goes well, the dye will appear in the arteries and glomeruli in a few seconds. Observe the blue particles in the walls of the renal tubules. If time permits observe the effects of injected glucose and of adrenalin.

A complete bibliography on the secretion of dye stuffs by the kidney will be found in the recent paper by Höber, Jour. Cell. Comp. Physiol., 6: 117, 1935. The comparative physiology of the kidney is reviewed by

Marshall, Physiol. Rev., 14: 133, 1934. Cf. also Evans' "Recent Advances in Physiology," 5th Ed., p. 377, 1936. Text pp. 246, 249, 263.

16. Perfusion of the Liver.—Secure a capillary cannula with a lip (must be made by a glass blower). If special cannulae are not available use cannulae described in Ex. 15a. Cut one side of the body wall of a frog. Lay back the body wall without injury to the vena abdominalis. Tie the cannula in an incision in the gall bladder. To exclude circulation from the hepatic portal vein a ligature must be tied tightly around the oesophagus and fold of intestine containing the pancreas. Perfuse the abdominal vein with 0.0005% eriocyanin (this dye can be obtained from K. Hollborn, Leipzig, through Pfaltz and Bauer, New York). The dye will emerge from the cannula in a 0.5% concentration within two hours (or the gall bladder will become blue) cf. Höber and Titajew, Arch. ges. Physiol., 223: 180, 1929. Text p. 249.

Directions for experiments 15 and 16 were prepared by R. W. Lippman.

XII. TEMPERATURE CHARACTERISTICS

(See Appendix of Text for Index to Temperature Characteristics)

In these experiments graphs must be plotted and the best straight line drawn through the points. Calculate μ from this line. Text p. 387.

1. CO_2 Production of Muscle.—Three test tubes are supported in holes drilled in a board resting on top of a thermostat jar. Add five drops of Phenol-red to standards and to the observation tube containing frog's muscle in 10 cc. Ringer. Determine time for pH to change from 7.6 to 7.4 at several temperatures from 15 to 25°. The standard buffer solutions can be made up to any fixed pH values in this range. **Constant temperaatures must be maintained.** Calculate the apparent temperature characteristic.

Begin experiment as soon as possible for the changes are slow. The central tube containing the muscle and Ringer's solution will be more alkaline than both standards but will change as CO_2 is given off. Replace Ringer for each temperature. The same muscle must be used. Large muscles produce most rapid changes (entire leg may be used). Keep tubes corked. Match tubes at same level cf. Crozier, J. Gen. Physiol., 7: 189, 1924.

2. Breathing Movements of Frog.—Determine the time for ten pharyngeal movements in a frog (large females are sedentary) at as many temperatures as possible between 7 and 28°C. Immerse the animal in an air tight preserve jar weighted with buck shot covered with paraffin. Cover thermostat with paper leaving peep-hole for observation. Allow 10 mins. for temperature adjustment. Avoid all possible disturbance. Remove the lid of jar at each change for renewal of air. Calculate the temperature characteristic with the Arrhenius equation.

$$ln \frac{\text{Rate at } T_2}{\text{Rate at } T_1} = \frac{\mu}{2}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)$$

Frequency may be expressed as

$$\frac{1}{\text{Time for ten beats}} \times 100$$

Plot log frequency against $\frac{1}{\text{T Abs.}}$. Cf. Crozier, J. Gen. Physiol., 7: 571, 1924–25. A critical temperature occurs at 15°C.

The traditional view is that these movements are controlled by H ion acting on the respiratory centre. "H ions" are revealed by a temperature characteristic of 20,000 calories but your value is much lower (5,000 — 8,000). Text p. 42.

3. Opercular Movements of Goldfish.—Place a goldfish in a beaker which fits in a wooden support on top of the thermostat jar. Determine the relation between temperature and opercular moments (5–28°C.). Calculate the temperature characteristic cf. Crozier, J. Gen. Physiol., 9: 552. The Arrheniuse quation is discussed in Taylor, "Elementary Physical Chemistry," 1927, p. 167. Reciprocal temperatures may be obtained from Fig. 8, p. 50, Buchanan and Fulmer, "Physiology of Bacteria," vol. 2, 1930. The μ value may be obtained without calculation from the nomogram of Richards, Jour. Phys. Chem., 30: 1219; Buchanan and Fulmer, vol. 2, p. 49. Text pp. 385–395.

XIII. RHYTHMICITY OF THE HEART

1. Automatic Rhythmicity of the Heart.—Expose the heart of a frog. Keep moist with Ringer. Determine rate and sequence of beats. Cut widely around the sinus venosus and the veins entering it, sever the two aortae, and thus separate the heart from the body. Place the excised heart in a watch glass. Do the contractions continue? If so, note the rate immediately after the excision and again a few moments later.

Cut between sinus and right auricle at the sinoauricular junction. Does the sinus continue to beat? (It will be necessary to wait until the immediate effects of cutting have disappeared.) Does the rest of the heart now beat? And if so, at what rate?

Cut between auricles and ventricle, just above auriculoventricular groove. Do contractions occur in the isolated parts?

Cut through the ventricle, just below the auriculoventricular groove. Does the apex beat? Stimulate it, by tapping it, and by a crystal of NaCl. According to Heinbecker and Bishop, Am. J. Physiol., 114: 212, 1935, the ventricle of the turtle is free of ganglia and therefore cannot beat.

2. The Stannius Ligature (Preparation for Ex. 3).—The automatic contraction of the ventricle can be inhibited by means of a "Stannius

Ligature." Moisten with Ringer a ligature thread and pass it under the two aortae. Lift up the ventricle (turn it back) and tie the thread exactly over the line marking the junction of the sinus and auricle. If the ligature is not exactly placed, no change may occur. In that case tie another thread at the same junction. Sometimes it takes 5 ligatures. What is the change produced by the tying? (cf. Mitchell, "Gen. Physiol.," 1932, p. 579). Compare with mammalian heart (cf. Wiggers, "Physiology in Health and Disease," 1934, p. 445). This preparation is to be used in Ex. 3. For a recent discussion of heart block cf. Gilson, Am. J. Physiol., 110: 376, 1935. Text 432, 434.

3. The All-or-None Effect in the Heart (Use a stationary drum).—The beat of the auricle and ventricle are inhibited by a ligature (Ex. 2). Fasten a **straight** fine wire (1 ft.) to one post of the secondary coil of the inductorium. Make sure that it springs freely in a vertical direction. Bend down about 3 ins. of the free end directly over the heart and bend a fine hook at the tip which is to be inserted into the tip of the ventricle. Now tie a thread on the wire at the bend and fasten the other end of the thread to the heart lever.

The following points must all be in the same vertical line directly above the heart: attachment of thread on lever; attachment of thread on wire; vertical arm of wire; attachment of hook to ventricle. Make careful adjustments so that the heart has full mechanical advantage.

THE HEART MUST BE DIRECTLY BENEATH ITS ATTACHMENT TO LEVER. Tie another inductorium wire at ligature.

Find a break shock that will cause the heart to contract. Record the contraction. Turn the drum 2 mm., slide out the secondary coil 1 mm., and after waiting 15 secs. stimulate again. Continue thus until the heart fails to respond.

Does the height of the contraction under these conditions vary with the strength of the stimulus? Cf. Bowditch, Ber. d. k. sachs. Gessell. d. Wiss., Leipzig, 1871, p. 682; Bayliss, p. 453; Barcroft, "Features in the Architecture of Physiological Function," 1934, p. 250. Text p. 426.

4. Treppe.—On a stationary drum record a series of contractions, at 2 sec. intervals, of a heart arranged as in Ex. 3, using break shocks of **constant** strength (cf. Bayliss, p. 453; Mines, Jl. Physiol., 46: 1, 1913). Compare with treppe in a nerve net (cf. Pantin, Jl. Exp. Biol., 12: 389, 1935). Text p. 342.

5. Changes of Irritability during a Single Cycle.—Arrange the heart of a fresh frog as in Ex. 3 **but without the ligature.** Introduce the wires only **into**, not through, the muscular wall of the heart; be careful to avoid loss of blood. Let a signal, **set as close as possible beneath the heart lever,** record the moment of stimulation. Start the drum at a rate which results in a widely separated record of systole and diastole. Study the

record with reference to the contraction. Is the contraction of the auricle shown in the tracing? See Rogers, p. 187, for normal heart record.

Stimulate the ventricle with minimal make or break shocks, but do not let the two stimuli fall within a single cardiac cycle. Try to stimulate at various stages in systole and diastole, but let normal beats intervene between those which are disturbed. By means of **simultaneous ordinates** determine the moment when the stimulus was applied. Marey discovered that the vertebrate heart is refractory during systole. Cf. Bayliss, p. 454. Why has the heart a long refractory period? According to Ritchie (Nature, 129: 165, 1932) the long refractory period of the heart permits it to recharge beyond the point of stability, hence the spontaneous excitation.

6. The Recovery of Excitability Following a Response.—In Ex. 3 you were cautioned to wait 15 seconds between successive excitations. The following experiment will show why.

Use the preparation described in Ex. 3, (with ligature). Insert a signal magnet in the primary circuit and arrange the inductorium so that the make shock is just adequate to excite. With the drum rotating very slowly stimulate with **pairs** of inductorium induction shocks, the make following the break at gradually decreasing intervals (3 seconds and less) until no response is obtained. The interval **between the pairs** of stimulations should be constant and at least 15 seconds in length. The second curve will drop out when the refractory interval is reached.

7. Measurement of Changes in Irritability (A tedious experiment).—Use a fresh preparation with a Stannius ligature. Start with the inductorium in a position such that the make shock is just effective when applied to the fully recovered muscle. Stimulate with a break shock followed by a make shock after an interval of 7 secs. Keeping the interval at 7 secs. increase the distance between the primary and secondary coil one mm. at a time until the make shock becomes ineffective. A rest interval of at least 15 secs. should separate the pairs of excitations. Record the position at which the make shock is least effective with a 7 sec. interval.

Repeat, reducing the interval between break and make to 6, 5, 4, 3, 2, and 1 secs., and fractionating the intervals further if you can do so. The intervals may be represented: break 7 secs., make—15 secs. wait—break 6 secs. make—15 secs. wait—break 4 secs. make—15 secs. wait, etc. At end of 7 sec. interval move coil to find threshold DISTANCE.

The heart becomes refractory for a certain time interval after the first shock. The position of the secondary coil for the second shock (make) measures the excitability at the particular time interval, i.e., 7, 6, 5, 4, 3, 2, 1 secs. after the initial beat. Immediately following the first stimulation no response is obtained on the second shock. At longer time intervals you are testing the recovery of the heart. Plot the positions of the secondary coil, which may be taken as a measure of the excitability of the

cardiac muscle, against the corresponding time intervals. Can you distinguish:

The Absolute Refractory Period?
The Relative Refractory Period?
The Period of Hyperexcitability?

Compare with Adrians experiment (J. Physiol., vol. 54, p. 1, 1920). Explain the normal rhythmicity of the heart (cf. Bayliss, Fig. 139, p. 455).

8. The Effect of a "Tetanizing" Current.—Record on a slowly turning drum the effect of stimulating the ventricle for a short interval (5 seconds) with the weakest effective tetanizing current. Can cardiac muscle of vertebrates exhibit true complete tetanus? The apparent tetanus you obtain is probably **contracture.**

9. Gaskell's Clamp and Heart Block.—Expose the beating heart of a frog with brain pithed. Draw the ventricle between the edges of the Gaskell clamp. Screw the edges carefully against the auriculo-ventricular junction.

Continue to increase the pressure very cautiously. A pressure will be found at which the ventricle does not respond to every beat of the auricle, i.e., the contraction wave does not always pass from auricle to ventricle through the partial block. The clamp depresses the rate at which a hypothetical substance recovers its power of transmission.

What numerical ratios are noted between the contractions of the auricles and the contractions of the ventricle, as the pressure is slowly increased, and again as the pressure is lessened. Cf. Bayliss, p. 681; Gilson, Am. J. Physiol., 110: 376, 1935. Text p. 432.

10. Engelmann's Incisions.—With small scissors cut about three-fourths across the ventricle alternately on the two sides, beginning just below the auriculoventricular junction. Thus the ventricle is transformed into a zig-zag strip. If the contraction wave is still conducted into the ventricle, note whether the wave passes over the strip. If no part of the ventricle is active, stimulate the base with a minimal induction shock. Next apply the same stimulus to the apical end of the strip. The waves of contraction should travel equally well in either direction (cf. Engelmann, Pflüger's Arch. 61: 275, 1895; Schmitt, Am. J. Physiol., 85: 332, 1928). Text p. 433.

11. Inhibition of the Heart by Vagus Stimulation.—Remove the lower jaw of a frog. Carefully pick away muscles in the two depressions on each side of the occipital region. Expose the vagus as it leaves the skull. (Fig. 8.) Explore with electrodes while dissecting. Arrange the heart to record its contractions clearly on a slowly revolving drum. Set in the primary circuit of an inductorium a simple key and an electromagnetic signal. Using a weak interrupted current, stimulate the vagus nerve; first

for 5 seconds with the weakest current producing any effect, next with a strong current for the same length of time, and finally with the strong current for a longer time. The signal should record directly below the writing point of the heart lever. Cf. Mitchell, 2nd Ed., p. 37. Text p. 437.

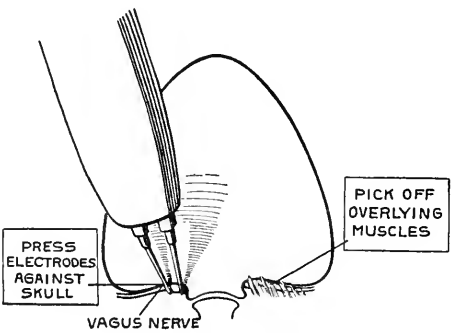

Fig. 8.—Stimulation of vagus nerve in the frog. Ventral aspect of skull. Lower jaw removed.

12. Influence of Temperature on the Frog's Heart.—Record on kymograph and count the frequency of the heart beats while Ringer solution of a known temperature is poured gently over the heart. Use several temperatures from 10° to 25°C. Calculate the "Temperature characteristic." Cf. Crozier, J. Gen. Physiol., 9: 531, 1926. If frog's heart could function at 37°C. would its frequency be similar to that of a warm blooded animal like the rabbit? Cf. Clark, "Comp. Physiol. of Heart," p. 40, 1927. Text p. 441.

13. Electrocardiogram of Frog.—Place excised frog heart on non-polarizable boot electrodes (Zn—sat. $ZnSO_4$) wired to the capillary electrometer. Observe the movement of the meniscus between the Hg column and the 20% H_2SO_4. Explain movement of Hg (by change in surface tension). Compare frequency of contraction with the electrical variation. Make a diagram of the instrument. The meniscus may be adjusted by the windlass at right. For description and limitations of capillary electrometer cf. Bayliss, p. 642. Try effect of M/8 Kll. M/12 $CaCl_2$ (cf. Clark, "Comp. Physiol. of Heart," p. 129).

EXPERIMENTS ON THE TORTOISE HEART

With a hook draw out the head. Smash the skull with screw driver and hammer. Pith brain with wire. Saw off the plastron. Slit the pericardium and expose the heart. Identify the different parts and trace the sequence of the contraction wave from the sinus to the bulbus arteriosus. Place the animal on its back in a pan. Keep neck extended by a cord passing under the pan and tied to a pelvic girdle. With pipette

draw off fluid in pericardium and determine volume. What is the function of pericardium? (cf. Howell, 12th Ed., p. 575; Skramlik, Zeit. vergl. Physiol., 15: 550, 1931).

14. Inhibition by Vagus Stimulation.—Expose the vagus nerves at the base of the neck and place silk ligatures loosely about them. The ligatures will be tied later. Arrange an induction coil for tetanizing currents of moderate strength, and, using the briefest possible periods of stimulation, make sure by physiological test that the nerves isolated are the vagi.

(a) Describe the appearance of the heart during vagal stimulation.

Attach one heart lever by a thread to tips of the auricles (thread with needle). Attach a second lever to the tip of ventricle. Arrange two heart levers to write on a smoked drum; set the writing points in the same vertical line, and directly beneath them place an electro-magnetic signal. Counter weight levers with plasticine so that the chambers hang vertically.

(b) While recording simultaneously the contractions of the auricles and ventricle, carry out the following procedures. Be sure to leave a sufficient interval of time after each step to permit a return to the former state.

Stimulate the left vagus.

Stimulate the right vagus.

Tie the ligature on the left vagus.

Tie the ligature on the right vagus.

Apply prolonged stimulation to the right vagus peripheral to the ligature.

Describe the results in detail (cf. Bayliss, p. 683).

Distinguish between inotropic and chronotropic effects.

Allow the heart to trace its recovery after inhibition.

The action of the vagus was discovered by Lower, 1669, Tractatus de Corde, London. He ligated the nerve and thought that he had blocked the flow of "spirits" to the heart (cf. Haldane and Priestly, "Respiration," p. 2). The effect of stimulating the vagus was studied by the Weber brothers in 1845 (cf. Bayliss, p. 683). Text p. 437.

15. Cardiac Tonus and Salt Effects.—While the heart is still in place, cut the two auricles, with the sinus attached, away from the ventricle. Leave in place the ligatures about the tips of the auricles, attaching one to an L-shaped glass rod, the other to a heart lever. Slit open the auricles with scissors to permit diffusion of salts. Set the glass rod and the auricular muscle in a beaker containing 250 cc. of Ringer with oxygen. Let the drum turn at a speed which just fails to cause a fusion of the records of the contractions. Replace the Ringer with 0.75% NaCl plus 0.01% $NaHCO_3$ (buffer) and make a continuous record. Note that after a time

the beats are superimposed on oscillations in the base line. This is a beautiful example of RHYTHMICITY (cf. Fig. 49 in Fulton's "Muscular Contraction"). For a discussion of auricular tone see Bayliss, p. 540 or Meek, Physiol. Rev., 7: 259, 1927; Fano, Beitr. z. Physiol., Ludwig's Festschrift, p. 287, 1887.

When the strip shows signs of exhaustion add to the solution 3 cc. of 1% $CaCl_2$. Is there any change in the activity? If the activity is well restored by the $CaCl_2$ replace the contents of the beaker with 250 cc. of 0.7% NaCl containing 3 cc. of 0.9% KCl. What is the effect of K?

Replace KCl with Ringer's solution. (This solution should maintain prolonged contractions.) Text p. 436.

16. Influence of Temperature on Turtle Auricles.—Make a preparation of the auricles as in Ex. 15 (but do not slit auricles). Place the 250 cc. beaker of Ringer containing the auricles and O_2 tube in a wooden support on a large jar (used as thermostat). Place a syphon in large jar to facilitate change of water for temperature change. A second jar (for replacement) should be adjusted to next temperature desired. Register contractions of the auricle on kymograph drum. Start at room temperature. Go down to 9° or 8°C (if possible). Check at some intermediate temperatures. Then slowly go above room temperature and continue to 25°. Take the time for ten beats. Choose temperatures distributed about as follows: 25°, 24°, 22.5°, 21°, 19°, 17°, 16°, 14°, 12.5°, 11°, 9.5°. Calculate the characteristic. Cf. Crozier, J. Gen. Physiol., 9: 543, 1925. The temperature graphs of hearts are remarkable for their absence of "breaks" or critical temperatures.

17. Effect of Adrenalin (Ex. 16 continued).—Apply adrenalin and repeat temperature experiment. Place 1 cc. of adrenalin chloride solution in 100 cc. of Ringer. Take 1 cc. of this solution and add to the 250 cc. of Ringer, in the beaker containing the heart. Do not prepare solution until needed. The adrenalin shifts the axis of the graph without changing the slope showing that the master reaction is not changed. Three well distributed temperatures will show the effect (cf. Crozier, J. Gen. Physiol., 9: 541).

18. Model of Mammalian Heart (Harvard Circulation Scheme).—Pump = left ventricle. Valve in inlet tube to pump = mitral. Valve in outlet tube = aortic. Bamboo cane (upper left) = resistance of small arteries and capillaries. Tubes between pump and bambo = arteries; those on distal side of this resistance = veins. Opening short circuit tube (with clamp) at right of bambo tube = dilation of the vessels. Rim of eccentric brass plate = intraventricular pressure curve of dog (periphery = fractions of sec. radii = mm. Hg. pressure).

Each revolution of the eccentric plate reproduces in the ventricular tube both the time and pressure relations of the ventricular cycle in the

dog. **Turn the handle** and observe the arterial pressure (right Hg manometer) and the venous pressure (left Hg manometer). Feel the pulse in the arterial tube (at right).

Now connect the side tube (to right of bamboo resistance) to a membrane manometer. Clamp off artorial manometer. Record pulse tracing on a kymograph drum with signal magnet, marking time in seconds. The record is identical with the pulse of the carotid artery of the dog (cf. Porter, Science, 21: 752, 1905).

XIV. INVERTEBRATE HEARTS

ANNELID HEART

The worms are the lowest class of animals in which any structure analogous to the heart is found. In the earthworm the circulation is closed and maintained by peristalic waves of the dorsal vessel (and contraction of aortic loops).

1. Direction of Wave in Heart of Earthworm.—Pin a worm (dorsal side up) in a dissecting pan. Observe the dorsal vessel through the skin along median dorsal line. Any correlation with peristallic wave in body muscles? At which end of the dorsal vessel does the wave start? Place a mm. rule beside the animal and determine the rate of conduction at 5°, 10° and 25°C. (Cover with water at desired temperature.) If the worm is cut in 3 pieces, does the direction change? (cf. Clark, "Comp. Physiol. of Heart," pp. 3–4).

2. Circulation in the Leech.—Curarize (inject 0.2 cc. 1% curare half hr. before experiment) a medicinal leech (this paralizes the body muscles but does not affect the vascular contractions). Avoid injecting alimentary canal. Each longitudinal vessel consists of a thin walled tube lying laterally in the body cavity, slightly to the ventral side. Immerse animal in Ringer's solution diluted to ⅓. Cut through lateral body wall just deep enough to pass through longitudinal muscles (cut through ventral edge of the yellow line present in some species). The vessel is now exposed along the whole length of the excision (bulging around the distended nephridial vesicles). Determine the rate and sequence on each side (cf. Gaskell, Trans. Roy. Soc., 205: 168, 1914). Text p. 435.

ACCESSORY HEARTS IN INSECTS

Insects have a feebly developed open circulatory system since oxygen is transported by the tracheae. The accessory leg hearts appear to be myogenic unlike the lymph hearts of the frog.

3. Leg Heart in Notonecta.—Place the insect dorsum down in a dry watch glass. Under the microscope observe the pulsating organ in the femorotibial joint (just below knee) of the swimming leg (during moments

of quiescence). Determine the rate. Repeat with excised leg (cf. Crozier and Stier, J. Gen. Physiol., 10: 479, 1927).

CRUSTACEAN HEART

In the lobster the heart consists of a sac of striated muscle with 4 ostia through which the blood enters.

Cut a window on the top of the carapace and attach heart lever with thread and a fine hook. Tie thread near fulcrum for greater leverage. As these hearts are perishable do not bother with signal magnet or timing device. Perfuse with Marine Crustacean Ringer (NaCl 0.5M, $CaCl_2$ 0.04M, KCl .01M). Record on lightly smoked drum.

4. Ca-K Paradox in Crustacean Heart.—Drop 0.5M $CaCl_2$ on the beating heart and note arrest in diastole (unlike vertebrate heart). Cf. Hogben, "Comp. Physiol.," p. 17, 1926. Irrigate with Crustacean Ringer until recovery and apply 0.5M KCl which favors systole. Irrigate with Ringer for recovery. Text p. 440.

5. Tetanus in Crustacean Heart.—Place fine wires from an inductorium at each end of the heart and obtain a record under weak tetanizing current. The brothers Weber (1846) noted that crustacean heart shows no refractory period and summation and tetanus can be easily obtained (cf. Clark, "Compar. Physiol. of Heart," p. 9). Compare record with that of tetanized frog skeletal muscle. Text p. 432.

MOLLUSCAN HEART

The circulation in Molluscs is much more highly developed than in Arthropods. In the clam (Venus) the heart consists of a large ventricle (through which the intestine runs) and 2 thin-walled auricles (all smooth muscle). For oyster heart cf. Walzl, Physiol. Zool., 10: 125, 1937.

Remove one valve of the shell and open the pericardial cavity (near the hinge). Attach the heart lever thread to ventricle with a fine hook. Place the clam in a finger bowl containing Marine Molluscan Ringer (NaCl 0.4M, $CaCl_2$ .005M, $MgCl_2$ .08M). Record the beat. Immediately after exposure the heart may remain temporarily at rest. Open several clams until a satisfactory heart is obtained. If clams are kept on ice they should be exposed to room temperature a few hours before experiment.

6. K Systole in Clam Heart.—Remove the Ringer from the bowl and obtain a record of the effect of dropping M/2 KCl on the heart. Now add M/2 $CaCl_2$ and note the change. Return the clam to Ringer for a recovery record (for ionic effect, cf. Clark, p. 135). For effects in oyster heart cf. Jullien and Morin, Jour. Physiol. et Path. Gen., 29: 446, 1931. Text p. 440.

7. Effect of Adrenalin on Clam Heart.—Note the augmentor action of adrenalin. In vertebrates the inhibitory actions of adrenalin on various tissues are as common as augmentor effects but in invertebrates only augmentor action has been described (Clark, p. 62).

NOTE: If fresh water mussels are studied use mussel Ringer (NaCl 0.12%, $CaCl_2$ 0.012%, KCl 0.015%, pH 7.8). Excess KCl arrests these hearts in diastole (cf. Motley Physiol. Zool., 7: 62, 1934). According to Kock, Pflügers Arch., 156, 1916, excess K or Ca causes systolic standstill in heart of Anodonta.

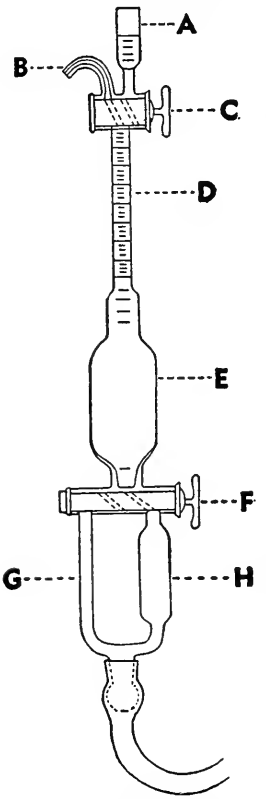

Fig. 9.—Blood-gas pump. A, cup for blood sample; B, outlet tube; C, stopcock for connecting D with A or B; D, upper stem of pipette marked with 0.02 cc. divisions; E, bulb of 50 cc. pipette; F, stopcock for connecting E with G or H; G, H, mercury trap for air which may leak in. The rubber tube at bottom is connected to a mercury leveling bulb.

DEMONSTRATION

Oscillograph Recording of Action Current in the Heart.—A frog's heart is placed on cotton points soaked in Ringer leading to non-polarizable Ag-AgCl electrodes connected to an amplifier which magnifies the potential changes recorded by an iron tongue oscillograph and loud speaker.

XV. COMPARATIVE PHYSIOLOGY OF RESPIRATORY PIGMENTS

Haemoglobin is the best-known of the pyrrole pigments of animals and consists of a protein portion, a dyestuff (prosthetic group) containing iron. The following directions are taken from Cannon's "Laboratory Course in Physiology," by kind permission of Harvard University Press. For a brief account of oxygen transporters in various animals cf. Florkin, "Transporteurs d'oxygène," Hermann, Paris, 1934; Roche, "Biochemie des pigments respiratoires," Masson, Paris, 1935. Text pp. 412–413.

1. Determination of Carbon Dioxide Dissociation Curve of Ox Blood.—A blood-gas pump has as an essential feature a reservoir which can be filled with mercury, and after its connections have been tightly closed can have a vacuum developed within it by the withdrawal of the mercury. A simple apparatus of this character is that devised by Van Slyke (Van Slyke, J. Biol. Chem., 30: 347, 1917). It consists essentially of a 50 cc. pipette (D, E) (Fig. 9) with a three-way cock at the top (C) and a one-way cock at the bottom (F), and a 1 cc. scale on the upper stem of the pipette (D) divided into 0.02 cc. divisions. Below the lower cock is a chamber (H) which serves as a trap for air which may leak in through the connection with the rubber tube below. This tube, which is of heavy-walled rubber, leads to a leveling bulb filled with mercury. The CO_2 content of blood is to be determined when it is in equilibrium with gas mixtures (air) containing CO_2 at several different pressures.

The use of tonometers is described by D. B. Dill in the Appendix of L. J. Henderson's book "Blood," 1928.

Tonometers in which the beef blood is to be equilibrated to CO_2 at partial pressures of 20, 40, and 100 mm. Hg are ordinarily prepared a day in advance. These tonometers are of the Barcroft type of 250 and 350 cc. and have the exact capacity etched on each one. The dry tubes are fitted with rubber stoppers. The stopcocks, which are well lubricated, are held in place by rubber bands. The procedure consists in removing a fraction of the air in the tonometer and replacing it with CO_2. If the capacities in cc. of the three tubes used are designated a, b, and c, then the volumes to be replaced with CO_2 are respectively 20a/B, 40b/B, and 100c/B where the numbers represent the partial pressures required, and B the barometric pressure in mm. Hg. The tonometer of volume a, for example, is connected to the gas pipette and the Hg lowered for the calculated volume, 20a/B. This air is expelled through the side arm of the pipette. The tonometer is disconnected and a bladder of CO_2 is attached to the side arm, and the apparatus flushed with CO_2. The proper amount of CO_2 is then drawn into the pipette, the tonometer connected and the gas run into it.

By means of a 10 cc. syringe 6 cc. of the beef blood is introduced into the tonometer through its stopcock. With another syringe mercury is forced through the stopcock until the capillary pores are filled.

The tonometer is held upright between the hands and gently agitated for 10 minutes. The stopcock is turned to the side arm, and the tonometer connected by a short tube to the outflow tube of a clean Van Slyke apparatus, the stopcock of which is turned to the outflow tube. The leveling bulb is raised until the mercury appears in the side arm of the tonometer. The stopcock of the latter is turned to connect with the chamber and 1.5 cc. of blood is drawn into the Van Slyke. The stopcocks are closed and the tonometer disconnected. Then the blood except exactly 1 cc. is expelled through the outlet tube.

1. Place 3 cc. of water in the cup. Insert a drop pipette to the bottom of the cup and, while 1 cc. of the water is allowed to flow into the burette, inject from the pipette about 4 drops of caprylic alcohol. The alcohol, thus washed into the burette, will prevent foaming.

2. By means of a drop pipette remove the excess water from the cup.

3. Run into the burette 0.5 cc. of 10 per cent lactic acid and close the cock.

4. Evacuate the bulb by lowering the mercury nearly to the lower cock. Turn the cock. Shake the remnant of mercury with the blood for 3 minutes. Immediately read the volume of the gas in the manner demonstrated. (The lower cock is opened, the mercury in the leveling

bottle is raised until it is above the level of the mercury in the burette by $\frac{1}{13}$ of the column of laked blood.)

5. With a negative pressure of several centimeters allow 2 cc. of 1.0 N NaOH to run in and absorb the CO_2 that has been liberated.

6. Wait one minute for drainage and read again the gas volume.

7. Repeat process 5 with 1 cc. of the NaOH until two check readings are obtained. Draw down 1 cc., shake, then take reading.

8. Wash the burette with distilled water.

The difference between the first and second readings is the volume of CO_2 in 1 cc. of blood at the room temperature and the existing barometric pressure. To correct to standard conditions of 0° and 760 mm. barometric pressure, multiply by a factor obtained from Table 2 on p. 112 which takes account not only of the effect of temperature and barometric pressure upon the gas volume but also corrects for the aqueous vapor present and for a small amount of CO_2 which remains unextracted or which is reabsorbed by the solutions during the analysis. Cf. Van Slyke and Stadie, J. Biol. Chem., 49: 1, 1921.

The determination should be repeated several times with the same gas mixture until reasonable checks are obtained. Then repeat, using two other pressures of CO_2. Plot the results with volumes per cent of CO_2 as ordinates and CO_2 pressures as abscissae. Draw a smooth curve from the origin and through the points as determined. Cf. L. J. Henderson, "Blood," p. 78. Text p. 414.

2. Calculation of the CO_2 in Solution and in Chemical Combination.—The volume of CO_2 dissolved by 100 cc. of blood at a temperature of 38°C. and at any partial pressure of CO_2 (p.p. CO_2) is given by the calculation: $100 \times 0.511 \times \frac{\text{p.p. } CO_2}{760}$ or $0.0672 \times$ p.p. CO_2 (0.511 being the coefficient of solubility of CO_2 in blood). This represents CO_2 as a dissolved gas and as carbonic acid. The remainder is present combined with base. Calculate the proportion of CO_2 in solution and in chemical combination with base at each of the pressures determined above. Draw a line on your graph indicating the volume per cent of CO_2 in solution at various pressures. Text p. 414.

3. Calculation of the pH of the Blood.—This may be calculated as follows:

$$\text{pH} = 6.1 - \log \frac{(\text{Vol. \% } CO_2 \text{ combined with base})}{(\text{Vol. \% } CO_2 \text{ in solution})}$$

Estimate the pH of the blood at each of the pressures measured above. Plot a curve relating the pH and the CO_2 pressure. What is the pH at the pressure of CO_2 in arterial blood (40 mm.)? in venous blood (about 50 mm.)?

For values of the pH of blood determined by the glass electrode cf. Haugaard and Lundsteen, Comp. rend. Labor. Carlsberg, Sér. chim. 21: 85, 1936.

4. Determination of the O_2 Capacity of Ox Blood.—This may be defined as the volume of oxygen which may be extracted from 100 cc. of blood when it is in equilibrium with atmospheric air. Place 2.5 cc. of blood in the Van Slyke apparatus, draw in atmospheric air and shake the apparatus for 3 minutes. It is not necessary to change the air. Drive out the air through the cup and fill the bore of the stopcock which is on the side of the cup with mercury from the small mercury bottle. Turn the upper stopcock and through the outflow tube run all the blood in excess of **exactly 2 cc.** Close the stopcock and rinse out the cup. Proceed with the determination as follows:

1. Place in the cup

   6 drops of 1% saponin
   2 drops of caprylic alcohol
   6 cc. of water

2. Run into burette **all but 1 cc.** Wait one minute for haemolysis to occur.
3. Add 3 drops of 20% potassium ferricyanide to the contents of the cup and run it in.
4. Seal with a drop of mercury.
5. Evacuate the bulb and shake the analyzer for 3 minutes.
6. Absorb the CO_2 with 0.5 cc. of 0.5 N sodium hydrate.
7. Wait 2 minutes for drainage. Read.
8. Wash the apparatus with distilled water.
9. Subtract 0.115 from the reading for air dissolved in the reagent. Correct the remaining figure for nitrogen dissolved in blood, and for aqueous vapor, barometric pressure and temperature (see Table 3 on p. 112). Calculate the per cent of haemoglobin.

Blood is said to contain 100 per cent haemoglobin when its O_2 capacity is 18.5 volumes per cent. The per cent of haemoglobin in the sample is consequently given by

$$\frac{O_2 \text{ capacity}}{18.5} \times 100.$$

5. Determination of the O_2 Dissociation Curve.—Gas mixtures will be provided containing a constant percentage of CO_2 and varying percentages of O_2. (Mixtures of 6% CO_2 and 4.5% O_2 and 2.5% O_2.) Calculate the partial pressure of each gas present in each mixture used.

In the Van Slyke apparatus equilibrate blood with these mixtures, taking care to change the gas mixture at least once as in determining the

CO_2 dissociation curve. Seal the outflow tube with mercury after admitting the final portion of the gas. Determine the O_2 content. Determine the oxygen capacity of the blood used, and estimate the percentage saturation of each sample, as follows:

$$\text{The percentage saturation} = \frac{\text{Content}}{\text{Capacity}} \times 100$$

Plot the percentage saturation as ordinates against the O_2 pressure and draw a curve from the origin through the points. Cf. Henderson, "Blood," p. 75; Bayliss, p. 621. Text p. 414.

6. Determination of the O_2 Capacity of Limulus Blood.—Read Redfield's paper (J. Biol. Chem., 69: 475, 1926) before attempting experiment. Blood may be drawn from the cephalothorax by inserting a cannula. A heavy agglutinum forms on standing. This can be removed by centrifuging or by shaking while agglutination is taking place and then filtering off the serum.

The following modifications of standard procedure are necessary.

Trap the fluid after extraction of the gas (to prevent trouble from agglutinum). Estimate correction for added reagents by measuring at each reading the height of the column of aqueous fluid in the burette (use the attached min. scale from 0.5 cc. mark downward). Multiply the difference in the height of the water column by 0.926 (gives pressure due to the Hg displaced less the fraction of this pressure due to the displacing water).

Decolorize the oxyhaemocyanin by introducing 5% KCN saturated with caprylic alcohol into the burette and remove dissolved gases. Run the sample into this solution. A fine white precipitate is produced from the haemocyanin which gives up its O_2 uniformly. Sodium hydrosulfite and 1N NaOH are used for reabsorbtion of CO_2 and O_2.

Plot the percentage saturation as ordinates against the O_2 pressures and draw a curve from the origin through the points. Compare with the haemoglobin curve. Cf. Henderson, "Blood," Fig. 208, p. 322. The chemical nature of haemocyanin is discussed in Harrow and Sherwin's "Biochemistry," pp. 504, 529, 1935. Text p. 416.

XVI. COMPARATIVE PHYSIOLOGY OF RESPIRATION

1. Human Respiratory Movements.—With chest in mid-way position, fasten the Fitz pneumograph by means of the chain. Cautiously connect tube to tambour and record normal respiratory movements (eupnea) on a kymograph drum (time marker, 5 secs). Record (a) the effect of breathing through a narrow tube (dyspnea); (b) forced breathing, (c) swallowing, (d) singing, (e) reading aloud, (f) sneezing (sniff pepper, snuff or cigarette smoke), (g) coughing, (h) hiccoughing, (i) belching,

(j) breathing into a paper bag, (k) effect of running up and down stairs. The subject must not watch the record. Identify all records. Cf. Haldane and Priestly, "Respiration"; Wiggers, "Physiology," chapt. 24; Howell, 12th Ed., p. 682. The pneumograph was devised by Fitz, Jour. Exp. Med., 1: 677, 1896.

2. Respiration Scheme (Harvard).—Central glass cylinder = thorax. Ballon = lung. Surface of water = diaphragm. Left manometer = intrathoracic pressure. Right manometer = intrapulmonary pressure. Right tube at top = trachea with opening (glottis). Left tube at top when open represents a pleural puncture. With left upper tube closed and right open (glottis) lower the bottle at left of apparatus and raise. Repeat. Make a diagram showing pressure in manometers during normal respiration. Open upper left (pleural) tube and repeat. Represent other features such as forced breathing, coughing, etc. (cf. W. T. Porter, Am. Jour. Physiol., 10: 42, 1904). For a discussion of intrapulmonary and intrathoracic pressure see Howell, 12th Ed., pp. 689–690.

3. Respiratory Exchange in the Frog.—Determine the volume of the bottle provided to contain the frog. Close with a tight fitting rubber with sealed outlet tube. Label the bottle with your name, date, and time. With the Haldane Henderson gas apparatus determine the O_2 and the CO_2 after several days. Weigh the frog and express the respiratory exchange per gram body wt. For procedure in gas analysis (cf. Cannon, Lab. Course, p. 134). Compare with other animals (cf. Rogers, p. 257). Half the O_2 intake is through the skin while the CO_2 elimination through the skin is 6 times that from lungs. The frog absorbs about 47 cc. O_2 per kilogram body wt. per hr. at 18.8 C. Cf. Joel, Zs. Physiol. Chem., 107: 231, 1919); Winterstein, "Handb. vergl. Physiol.," vol. 2 Zweite Hälfte, p. 915, 1924.

4. Rectal Respiration in Dragon-fly Nymph.—The caudal part of the alimentary canal constitutes a tracheal gill. Water is taken in and forced out through the anal opening. Place a little carmine powder on the bottom of a small dish containing the naiad in water. Observe respiratory movements (underside of abdomen). Note movement of valves. Determine the rate. Bubble CO_2 through water vigorously. Now note the rate and force. Explain. Cut off the legs and place animal in a large dish of water. Tap abdomen (with a glass rod) and observe propulsive action of the rectal mechanism.

Cut off head. Note **rate, regularity** and **force** of respiratory movements. Cut off abdomen (just behind attachment of wing rudiments). Repeat observations.

Now insert point of scissors in anus and cut through upper abdominal wall. Pin open alimentary canal in paraffined watch glass and observe under binocular microscope or with lens. Note mass of tracheal tubes and

fluttering of valves. For description of gill see Comstock, "An Introduction to Entomology," 1920, pp. 319–320.

5. Respiration in the Giant Water-Bug (Lethocerus).—Observe the air tube at caudal extremity. Air is stored under the wing covers over the tracheal openings (Comstock, p. 366). Sketch the animal at the surface.

6. Respiration of Unicellular Organisms.—Place yeast suspension in the Warburg-Barcroft (or other type of respirometer). Place a small roll of filter paper soaked with 20% KOH in small vial to absorb CO_2. Measure the rate of O_2 consumption (cf. Dixon, "Manometric Methods," 1934). A differential volumeter is described by Fenn, Am. Instrument Co., Washington, 1935.

TABLE 1.—BUFFER MIXTURES

Sørensen's Phosphate Buffer Mixtures

Secondary ..... 11.876 g. $Na_2HPO_4\ 2H_2O$ per l.

Primary ..... 9.078 g. KH_2PO_4 per l.

Secondary phosphate Na_2HPO_4, cc.	Primary phosphate KH_2PO_4, cc.	pH
0.25	9.75	5.288
0.5	9.5	5.589
1.0	9.0	5.906
2.0	8.0	6.239
3.0	7.0	6.468
4.0	6.0	6.643
5.0	5.0	6.813
6.0	4.0	6.979
7.0	3.0	7.168
8.0	2.0	7.381
9.0	1.0	7.731
9.5	0.5	8.043

McIlvaine's Standard Buffer Solutions

(Shake after Mixing)

pH	0.2 M Na_2HPO_4, cc.	0.1 M citric acid, cc.
2.2	0.40	19.60
2.4	1.24	18.76
2.6	2.18	17.82
2.8	3.17	16.83
3.0	4.11	15.89
3.2	4.94	15.06
3.4	5.70	14.30
3.6	6.44	13.56
3.8	7.10	12.90
4.0	7.71	12.29
4.2	8.28	11.72
4.4	8.82	11.18
4.6	9.35	10.65
4.8	9.86	10.14
5.0	10.30	9.70
5.2	10.72	9.28
5.4	11.15	8.85
5.6	11.60	8.40
5.8	12.09	7.91
6.0	12.63	7.37
6.2	13.22	6.78
6.4	13.85	6.15
6.6	14.55	5.45
6.8	15.45	4.55
7.0	16.47	3.53
7.2	17.39	2.61
7.4	18.17	1.83
7.6	18.73	1.27
7.8	19.15	0.85
8.0	19.45	0.55

TABLE 2.—CORRECTION FACTORS FOR Co_2 DETERMINATION
(Cf. Van Slyke and Stadie, J. Biol. Chem., 49: 1, 1921)
Bar. = Barometric Pressure

Temperature (°C.)	Factor
15	1.002 × Bar./760
16	0.995 × Bar./760
17	0.989 × Bar./760
18	0.983 × Bar./760
19	0.978 × Bar./760
20	0.972 × Bar./760
21	0.966 × Bar./760
22	0.960 × Bar./760
23	0.954 × Bar./760
24	0.948 × Bar./760
25	0.942 × Bar./760
26	0.936 × Bar./760
27	0.931 × Bar./760
28	0.924 × Bar./760
29	0.918 × Bar./760
30	0.912 × Bar./760

TABLE 3.—FACTORS FOR CALCULATING RESULTS FROM ANALYSIS OF 2 CC. OF BLOOD SATURATED WITH AIR
Bar. = Barometric Pressure

Temperature, °C.	Air physically dissolved by 2 cc. of blood. Subtract from gas volume read in order to obtain corrected gas volume, representing O_2 set free from haemoglobin, cc.	Factor by which corrected gas volume is multiplied in order to give oxygen chemically bound by 100 cc. of blood, cc.
15	0.037	46.5 × Bar./760
16	0.036	46.3 × Bar./760
17	0.036	46.0 × Bar./760
18	0.035	45.8 × Bar./760
19	0.035	45.6 × Bar./760
20	0.034	45.4 × Bar./760
21	0.033	45.1 × Bar./760
22	0.033	44.9 × Bar./760
23	0.032	44.7 × Bar./760
24	0.032	44.4 × Bar./760
25	0.031	44.2 × Bar./760
26	0.030	44.0 × Bar./760
27	0.030	43.7 × Bar./760
28	0.029	43.5 × Bar./760
29	0.029	43.3 × Bar./760
30	0.028	43.1 × Bar./760

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